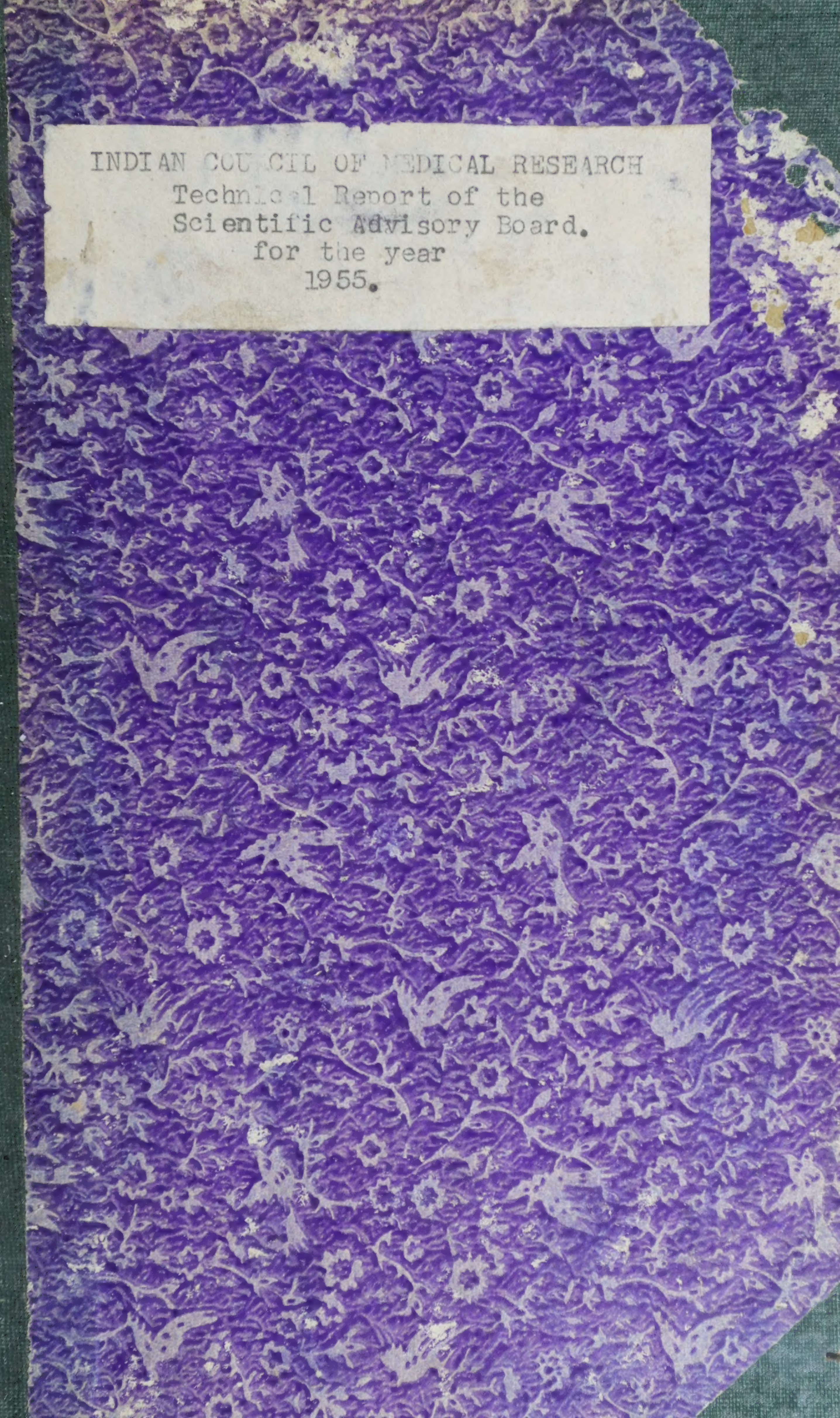

# INDIAN COUNCIL OF MEDICAL RESEARCH
# Technical Report of the Scientific Advisory Board.
for the year
1955.

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Indian Council of Medical Research

TECHNICAL REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR

1955

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Dr. Subodh Mitra, Director, Chittaranjan Cancer Hospital, 148, Russa Road, Calcutta, 26. (*Chairman*).

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Dr. (Smt.) S. Bhatia, Adviser in Maternity and Child Welfare, Directorate-General of Health Services, New Delhi.

Dr. C. R. Das Gupta, In-charge, Department of Hæmatology, School of Tropical Medicine, Calcutta.

Dr. V. S. Mangalik, Professor and Head of the Department of Pathology and Dean, Faculty of Medicine, K. G. Medical College, Lucknow.

Shri K. K. Mathen, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. T. B. Panse, Tata Memorial Hospital, Bombay.

Dr. (Smt.) H. S. Sharma, Assistant Director of Public Health, Madras.

Dr. R. K. K. Tampan, Director, Upgraded Department of Obstetrics & Gynæcology, Government Hospital for Women & Children, Egmore, Madras.

Dr. (Smt.) Muktha Sen, Professor of Maternity and Child Welfare All-India Institute of Hygiene & Public Health Calcutta. (*Secretary*)

VI. Mental Health.

Dr. M. V. Govindaswamy, Director, All-India Institute of Mental Health, Bangalore-2. (*Chairman*)

Dr. T. K. N. Menon, Dean, Faculty of Education and Psychology, M. S. University, Baroda.

Dr. S. C. Mitra, Head of the Department of Psychology, University College of Science and Technology, 92, Upper Circular Road, Calcutta.

Dr. L. D. Sanghvi, Research Officer, Indian Cancer Research Centre, Parel, Bombay-12.

Dr. Sohan Lal, Chief Pyschologist, Psychological Research Wing, Defence Science Organization, Ministry of Defence, New Delhi.

Dr. N. S. Vahia, Honorary Psychiatrist, Seth G. S. Medical College & K. E. M. Hospital, Bombay.

VII. Nutrition.

Dr. K. C. Sen, Director of Dairy Research, Government of India, Ministry of Food and Agriculture, New Delhi. (*Chairman*)

Dr. S. T. Achar, Professor of Pediatrics Madras Medical College & Government General Hospital, Madras.

Dr. A. C. Banerjea, 31, Station Road, Lucknow.

Dr. B. C. Guha, Head of the Department of Applied Chemistry, University College of Science & Technology, 92, Upper Circular Road, Calcutta.

Dr. K. Mitra, Assistant Director-General of Health Services, New Delhi.

Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor.

Dr. M. V. Radhakrishna Rao, Assistant Director, In-charge of the Department of Nutrition, Government of Bombay, Haffkine Institute, Parel, Bombay-12.

Dr. D. Subba Rao, Director of Public Health Andhra, 10, Sterling Road, Nungambakkam, Madras-6.

Dr. V. Subrahmanyan, Director, Central Food Technological Research Institute, Mysore.

Dr. K. Rajagopal, Professor of Biochemistry and Nutrition, All-India Institute of Hygiene & Public Health, Calcutta. (*Secretary*)

VIII. Physiology and Pharmacology.

Dr. B. Mukerji, Director, Central Drug Research Institute, Lucknow. (*Chairman*)

Dr. B. K. Anand, Professor of Physiology, Lady Hardinge Medical College New Delhi.

Dr. M. D. Chakravarty, Director, Central Drugs Laboratory, 6, Kyd Street, Calcutta.

Dr. B. B. Dikshit, Surgeon-General with the Government of Bombay, Contractor's Buildings, Nicol Road, Bombay-1.

Dr. Govinda Achari, Professor of Pharmacology and Therapeutics, Medical College, Patna.

Shri P. M. Nabar, Drugs Controller (India), Directorate-General of Health Services, New Delhi.

Dr. J. D. Pathak, Professor of Physiology, Medical College, Baroda.

Dr. (Smt.) Ranita Aiman, Professor of Pharmacology, B. J. Medical College, Poona.

Dr. K. S. Sanjivi, Professor of Medicine and Physician, Madras Medical College and Government General Hospital, Madras.

Dr. G. K. Karandikar, Professor of Pharmacology, Medical College, Baroda. *(Secretary)*

IX. Virus Diseases.

Lieut.-Colonel M. L. Ahuja, Director, Central Research Institute, Kasauli. *(Chairman)*

Dr. D. D. Banker, Assistant Professor of Pathology and Bacteriology, Seth G. S. Medical College, Bombay.

Dr. C. G. S. Iyer, Senior Research Officer, Neuropathological Unit, Indian Cancer Research Centre, Parel, Bombay-12.

Lieut.-Colonel S. L. Kalra, Assistant Professor of Pathology, Armed Forces Medical College, Poona.

Dr. J. A Kerr, Director, Virus Research Centre, Wellesley Road, Poona.

Dr. I. G. K. Menon, Observer, Government of India Influenza Centre, Pasteur Institute of Southern India, Coonoor.

Dr. T. Ramachandra Rao, Assistant Director of Public Health (Malaria) Bombay, Connaught House, Poona.

Dr. J. B. Srivastava, Professor of Pathology and Bacteriology, Medical College, Nagpur.

Dr. R. Sanjiva Rao, Director, Lymph Vaccine Institute, Belgaum. *(Secretary)*

III. TECHNICAL REPORT OF THE RESEARCHES CARRIED OUT DURING THE YEAR 1955.

The researches carried out during the year under report were recommended by the Scientific Advisory Board at its meetings held in Baroda on the 21st and 22nd December, 1954 and were approved by the Governing Body of the Indian Council of Medical Research at its meeting held in New Delhi on the 28th March, 1955. *The views expressed by the individual workers are not necessarily the views of the Council.*

CHOLERA

1. Inquiry on the mutation of cholera vibrios in fish under Dr. M. N. Lahiri at the All-India Institute of Hygiene & Public Health, Calcutta.

The objects of the inquiry were to study the possibility of mutation of cholera vibrios in the guts of fishes and to determine the serology of NAG suspected to be mutants, as also the phage typing of the original *V. choleræ* cultures and NAG.

Artificial infection experiments.—During the year under report ten series of artificial infection experiments were conducted with four species of hardy fishes, viz., the climbing perch (*Anabus testudineus*), the cat fishes (*Heteropneustes fossilis*) and (*Clarias magur*) and the murrel (*Ophicephalus punctatus*). Specimens obtained from their natural habitats were kept under observation in the laboratory till they ceased to excrete vibrios. It was found that these species of fish continued to excrete vibrios for a period of 4 to 5 months. This corroborates the observations reported last year regarding the natural excretion of NAG vibrios by the fish.

In artificial infection experiments, specimens rendered vibrio-free for a period of about 5 months were infected with both Ogawa and Inaba sub-types of *V. choleræ*. All the specimens excreted agglutinating vibrios for a period of three to four days subsequent to the infection. In each case, they excreted NAG vibrios for varying periods of time, within a maximum of 15 days after infection. The NAG excreted in the initial stages invariably belonged to Heiberg's group VI. In a few cases NAG of groups I and II were excreted after a few days but it is interesting to note that in every case the NAG excreted vibrios of this group only.

With a view to ascertain whether artificial infection with cholera vibrios would stimulate the excretion of NAG vibrios which may be retained in the guts of fishes presumed to be vibrio-free in the experiments, artificial infection was tried with other pathogenic oragnisms, viz., *Salmonella typhi* and *Sh. flexneri*. But none of the infected fish excreted any NAG vibrios. These experiments lend further support to the surmise that *Vibrio choleræ* may actually be undergoing some change in the guts of fishes.

Serological studies of NAG vibrios of group VI isolated after artificial infection were conducted. It was observed that the group VI vibrios obtained from the same species of fishes were serologically identical. But the same group isolated from different species showed serological differences. Similar studies of groups I and II vibrios are now in progress. Further work will be required to understand the significance of this finding.

Modification of the technique of isolation.—It was reported last year that comparative studies of culture techniques have indicated that the cultures should be incubated for 48 to 72 hours to obtain satisfactory results. Further studies were made during the year under report using cholera stools from cases admitted in the N. R. Sarkar Medical College Hospital as also water and gut content samples from fish. Cultures which were negative after 24 hours' incubation showed good growth of vibrios after 48 and 72 hours' incubation. A pH of 8·4 was found to be most suitable for the growth and survival of vibrios. In view of these findings it will now be necessary to re-examine the data collected during previous years regarding the absence of cholera vibrios in different sources.

Phage studies.—The phage typing of the vibrios is under progress and when a sufficient number of pure line phages have been obtained these will be utilized to study whether there is any relationship between the parent agglutination strains and the NAG strains obtained after artificial infection.

2. Inquiry into the state of anuria and uræmia in cholera with special reference to its treatment under Dr. A. Das at the Nilratan Sircar Medical College, Calcutta.

A series of 20 normal controls (healthy medical students) were investigated with a view to determine the normal values of sodium, potassium and chloride in plasma. The results are as follows:—

	In meqL Highest	In meqL Lowest	In meqL Average
Na ...	140·8	137	139·1
K ...	5·1	4·6	4·9
Cl ...	94·6	88·7	90·1

The cases of uræmia selected for this investigation were from the following groups:—

(A) Cases of cholera admitted with a degree of dehydration and collapse (radial pulse imperceptible) which suggested the possibility of development of uræmia. They had no clinical manifestations of uræmia at the time of admission—60 cases.

(*i*) Those that did not develop uræmia subsequently—42 cases.

(*ii*) Those that developed uræmia—18 cases (10 deaths).

(B) Similar cases but admitted with persistent anuria for at least 25 hours—5 cases (1 death).

(*i*) Admitted with clinical manifestations of uræmia—1 case.

(*ii*) Developed uræmia subsequently—4 cases.

(C) Those who developed obvious clinical manifestations of uræmia during treatment—22 cases (18 cases from group A and 4 cases from group B).

Thus, the total number of uræmia cases investigated was 23 including the case admitted with clinical manifestations of uræmia.

All the cases had the usual clinical manifestations of uræmia in varying degrees. One feature of interest was the presence of extra-cellular over-hydration developing during the course of treatment in 14 cases of this series. This was clinically manifested in six cases by engorgement of the veins of the neck (rise of venous pressure was confirmed by manometric measurements), puffiness on the eye-lids, sacral œdema and rise of body-weight. Over-hydration in each of these six cases was confirmed by fall of plasma specific gravity and hæmatocrit values. In eight other cases the presence of extra-cellular over-hydration was evident from the reduction of the plasma specific gravity and hæmatocrit values although the clinical manifestations of over-hydration had not yet appeared.

Biochemical findings in cases which were clinically diagnosed to be in a state of uræmia:

Sodium and potassium content of plasma.—Taking normal sodium of plasma to be between 130 and 143 meqL and normal potassium content to be between 4·1 and 5·6 meqL (Hawk *et al.*, 1947, ' Practical Physiological Chemistry ').

	Cases.
1. Normal sodium with	
(*i*) Normal potassium in plasma ...	7
(*ii*) Low potassium in plasma	1
2. High sodium with	
(*i*) Normal potassium in plasma ...	4
(*ii*) High potassium in plasma ...	1
3. Low sodium with	
(*i*) Normal potassium in plasma ...	8
(*ii*) High potassium in plasma ...	2
Blood urea	
(*i*) Below 70 mg. per cent	4
(*ii*) Between 71 and 120 mg. per cent ...	14
(*iii*) Above 120 mg. per cent ...	5

Plasma bicarbonate content

Between 22·4 c.c. and 43·68 c.c. of CO_2/100 c.c. of plasma in all cases (normal bicarbonate content of plasma, 53 c.c. to 75 c.c. of CO_2/100 c.c. of plasma).

Blood inorganic phosphate content

Between 4·5 and 17·6 mg. per cent (normal inorganic phosphate content of blood 3·7 mg. per cent).

Synopsis.—The concentration of sodium, potassium and chloride in the plasma of 20 normal controls (healthy medical students) was estimated. Twenty-three cases of uræmia in cholera were investigated. Twenty-two of these developed uræmia during the course of treatment, one manifested the clinical features of uræmia on admission. The study showed extra-cellular over-hydration in 14 of these cases. This points towards over-correction of the deficiency of body fluids during the anuric stage. Sixteen cases manifested deviations in the concentration of plasma Na and K. The plasma bicarbonate level was lowered in all cases confirming a state of acidosis. The mortality was high. Eleven out of the 23 cases died.

3. Inquiry on the evaluation of the phages acting on vibrios and the application of bacteriophage typing in epidemiological investigations on cholera under Dr. B. Ghosh Ray and Dr. M. N. Lahiri at the All-India Institute of Hygiene & Public Health, Calcutta.

Object.—To isolate different phages from cholera stool and study their lytic effect on *V. choleræ* and non-agglutinable (NAG) vibrios. This is essential to evolve a phage typing method which may be helpful in epidemiological investigations of cholera. The use of phages will also be made to elucidate the interrelationship between the *V. choleræ* and other NAG vibrios usually excreted by cholera cases.

Synopsis of work done.—Preliminary work was done on the standardization of media for isolating *V. choleræ* including the phage-carrying strains and NAG vibrios from cholera cases.

In order to find a simple way to handle large number of fæcal specimens either in hospital or in field practice, rectal swabs obtained either directly from patients or dipped in catheter specimens of the liquid stool, were employed for inoculating media. It was observed that even during the acute stage of the disease, simultaneous use of the bile-salt agar plates (pH 8·4) and the Wilson and Blair's liquid medium increased the chance of positive isolations. Two hundred and sixty samples of stool were examined. By direct plating method positive isolations were obtained from only 45 per cent of the samples. On the other hand, the combined method gave 58·8 per cent positive isolations. The effects of pH in Wilson and Blair's liquid medium, the period of incubation and the volumes of the media and of the inoculum are being studied further. The technique will be tried during the next recrudescence of the disease.

Swabs dipped in catheter specimen yielded positive isolations in 61 per cent cases, whereas the rectal swabs obtained directly were positive in 55 per cent cases. With further improvement in the technique, it is possible that employment of rectal swabs alone will be of great help. The strains of *V. choleræ* isolated during the last epidemic have been found to be of Inaba sub-type only. These and other NAG vibrios are being maintained for future investigations.

Fifteen phage strains have been obtained from cholera stools. Strains of *V. choleræ* including those carrying phages are being studied for further isolations. Experiments are in progress to obtain 'pure' line phages and determine their nature. So far, all the phages from stool filtrates are being propagated in association with the corresponding strain of *V. choleræ* isolated from the same samples of stool.

A start has been made to find out a convenient method to lyophilise the large number of vibrio strains already isolated as well as the phage preparations.

MALARIA

1. Insecticide and mosquito repellent inquiry under the Director, Malaria Institute of India, Delhi.

A. LABORATORY INVESTIGATIONS.

During the period under review, investigations on the following problems were carried out:—

1. Solubility of DDT crystals in the cuticular wax of houseflies and mosquitoes (Continued).
2. Investigations on the development of resistance in house-flies and mosquitoes to chlorinated hydrocarbon insecticides (continued).
3. Development of sporogony cycle of malaria parasites in resistant and non-resistant strains of mosquitoes after exposure to DDT (continued).
4. Prevention of sorption of insecticides into mud surfaces (continued).
5. Efficacy of newer insecticides such as Malathion and Diazinon.

B. FIELD INVESTIGATIONS.

Comparative effectiveness of DDT, BHC, Dieldrin and Malathion, residual sprays against mosquitoes.

C. SAMPLES OF INSECTICIDES AND SPRAYING EQUIPMENT TESTED.

D. PUBLICATIONS.

E. PROPOSED PLAN OF WORK 1956–57.

A. LABORATORY INVESTIGATIONS.

1. *Solubility of DDT crystals in the cuticular wax of house-flies and mosquitoes. Physical properties of cuticular lipoids.*

It was observed that the dissolution of DDT crystals in the cuticular wax of *M. nebulo* and *A. stephensi* started almost immediately and the majority of the crystals disappeared in three and six hours, respectively. In the case of *C. fatigans* crystals were observed intact even after 24 hours. This was considered interesting because these observations are in conformity with the natural biological tolerance of *Culex fatigans* to DDT residual deposits. The cuticular waxes of *M. nebulo* and *C. fatigans* extracted from the body (without wings) and from the legs were tested for their physical properties. The colour of the waxes from the legs was dark and that from the body dark brown. The various physical properties were as follows:—

Physical properties	*MUSCA*		*CULEX*	
	Body	Legs	Body	Legs
Slipping point	42°C.	55°C.	51°C.	60°C.
Melting point	45°C.	60°C.	52°C.	65°C.
Solidification	40°C.	53°C.	50°C.	58°C.
Refractive index ...	1·3852	1·4121	1·4512	1·4623

It was evident from data obtained that the waxes from the legs of both flies and mosquitoes melted at higher temperatures than the waxes from the body and those extracted from *Culex* had a higher melting point than the one extracted from *flies*. It is possible that this physical property is responsible for the natural biological resistance of *Culex* to DDT.

2. *Investigations on the development of resistance in house-flies and mosquitoes to chlorinated hydrocarbon insecticides.*

Reports from some parts of the world allege that in certain areas where DDT has been in use for a number of years, some species of mosquitoes have developed resistance to DDT and other allied insecticides. This has drawn the attention of all public health workers to the possibility of such a phenomenon interfering with the nation-wide programmes for the control of malaria by DDT used as a residual insecticide.

From the reports of successful malaria control obtained in various parts of India by DDT residual spray, it is concluded that so far anophelines vectors of malaria in India have not developed any appreciable degree of resistance although DDT has been in use in certain areas for over eight years. In order to study whether or not even a small amount of resistance has so far developed in some of the species, specific investigations were carried out in Northern India (States of Delhi and U.P.).

A. culicifacies collected from an area sprayed with DDT for over seven years was subjected to tests in accordance with the procedure laid down by the World Health Organization (Technical Report Series No. 80). These mosquitoes were found to be as susceptible to DDT as those from an unsprayed area. From the complete absence of *A. fluviatilis* and the absence of malaria transmission in sprayed areas in which investigations were carried out it is safe to conclude that no resistance has so far developed in U.P. Terai. Similar investigations are being carried out on other vector species in other parts of the country. In order to get as much information as possible within a very short time details of this technique and a testing kit has been forwarded to various workers for detecting if any resistance has developed in areas where DDT or other chlorinated hydrocarbon insecticides have been in use for a number of years.

Unlike anophelines, *Culex fatigans* have been found to have developed a certain degree of resistance in some parts (Delhi, U.P., and Madras). Studies on this problem are being continued.

The susceptibility of normal *A. culicifacies*, *A. subpictus*, *Culex fatigans* and *house-flies* to DDT has been determined so as to get a base line data which may be useful for purposes of comparison with similar data obtained from areas sprayed with DDT. The results are tabulated as under :—

Per cent mortality after one hour exposure*.

Species	Concentration of DDT			
	0·25 per cent	0·5 per cent	1·0 per cent	2·0 per cent
A. culicifacies … …	45·0	65·9	97·4	100
A. subpictus … …	46·6	61·1	94·8	100
House-flies … …	73·7	75·7	89·4	95·4
Per cent mortality after 24 hours exposure.				
C. fatigans … …	16·5	23·6	74·2	91·4

*The Probit-log and MLC$_{50}$ will be worked out when the observations are complete.

Laboratory investigations on the development of DDT resistance in *Culex fatigans* and *A. fluviatilis* have been continued in the Coonoor laboratories.

C. fatigans.—Signs of resistance first became apparent in the 14th generation. There was progressive increase of resistance in the subsequent progeny. The resistant strain has so far gone through 45 generations and the level of resistance has become very high. While the normal strain of *C. fatigans* evidenced 100 per cent mortality on being exposed to 200 mg. of DDT per sq. ft. for one hour, the resistant strain showed little or no mortality on being subjected to similar treatment under identical conditions. Adults of the resistant strain were resistant not only to mortality but also to the paralysing effect of DDT. The resistant strain showed striking variations in its tolerance for or resistance to DDT. With the dosage remaining the same, some individuals in a given batch of the females of the same generation, died after six hours of exposure, others after 12 hours and still a few others required even more than 18 hours of exposure to die.

A. fluviatilis.—Resistance as already reported was very late in appearing and very slow in building up. Feeble resistance was first evidenced in the 29th generation. The strain has so far progressed through 48 generations. There has been a slight increase in resistance but it still remains low. Degree of quantitative resistance in *A. fluviatilis* will be communicated at the time of the Indian Council of Medical Research meeting.

Similar studies have been carried out on the 4th instar larvæ of *Ædes ægypti* and *Culex fatigans.* The larvæ were exposed to DDT concentrations of 0·01 to 5·0 parts per million and BHC gamma isomer concentrations of 0·1 to 1 part per million. The results with *Ædes ægypti* are fairly consistent and the M.L.D. 50 for DDT and BHC appear to be about 0·02 ppm. The results with *Culex fatigans* are variable and need further confirmation.

Besides the above, it is also being investigated as to whether (1) the houseflies and mosquitoes exposed to DDT and BHC separately and to DDT and in BHC combination would develop resistance to DDT and BHC simultaneously; and (2) whether the development of resistance will be hastened if the same insecticide is used both as a larvicide and as an adulticide?

Selection of *Culex fatigans* and *Musca nebulo* to DDT, to BHC and DDT and BHC in combination has progressed to 28th and 33rd generations, respectively. It appears that when insects are exposed to combination of insecticides, the insects develop resistance not only to both the insecticides but also to individual insecticides simultaneously. Similarly, insects exposed to DDT or BHC separately also seem to develop certain degree of cross resistance.

3. *Development of sporogony cycle of malaria parasites in resistant and non-resistant strains of mosquitoes after exposure to DDT.*

Very little is known as to what happens to oocysts or to sporozoites in the body of resistant and normal strains of mosquitoes after exposure to DDT. Studies were undertaken at Coonoor on this problem and a few limited observations on the viability of gametocytes in fowls poisoned by oral administration of DDT were also recorded.

A DDT-resistant and a normal strain of *C. fatigans* for *P. relictum* in local sparrows, a DDT-resistant and a normal strain of *A. fluviatilis* for human malaria and only a normal strain of *Ædes (Stegomyia) ægypti* for *P. gallinaceum* in domestic fowls were used.

From the data available, it is evident that mere development of insecticidal resistance which was high in *C. fatigans* and low in *A. fluviatilis*, did not result in any concomitant change of the vectorial capacity of these two species.

In the case of *Ædes ægypti* also—it was seen that a sublethal exposure to DDT preceding or succeeding infective blood meal had no effect on the development of sporogony cycle of *P. gallinaceum* or on the sporozoites. Sporozoites in infective *Ædes ægypti* were not rendered non-infective by a sublethal contact with DDT, nor were impaired antigenically when sporozoite agglutination was studied.

Experiments with fowl indicate that in a normal fowl poisoned by DDT, the parasites multiplied and produced viable gametocytes which on being ingested by mosquitoes resulted in the production of sporozoites which, in turn, were proved to be infective to normal fowls.

4. *Prevention of sorption of insecticides into mud surfaces.*

(*a*) Correlation between the amount of insecticides sorbed into mud surfaces, to the porosity of the surfaces was studied. Panels with varying porosity ranging from 9·2 to 19·2 per cent were prepared by mixing different quantities of sand with mud and were dried in shade for a week under identical conditions. Dried panels were sprayed with 75 per cent DDT water-dispersible powder at the rate of 100 mg. DDT per sq. ft. The panels were allowed to dry for 24 hours after which scrapings from 25 sq. cm. surfaces were removed and the amount of DDT present in them determined by using a spectrophotometer. The results are briefly summarized below:—

Porosity	Residual DDT in scraping	DDT mg./sq. ft.
9·20	2·44	87·84
10·65	2·28	81·88
12·38	2·22	79·92
14·15	1·89	68·04
17·43	1·80	64·80
18·90	1·78	64·08
19·20	1·76	63·37

From the above table it would appear that absorption of DDT into mud increased with the increase in porosity. Further work is in progress on panels with the same chemical composition but of different porosity.

(*b*) *Studies on mud blocks received from Afghanistan.*—The World Health Organization sent a number of mud blocks from Afghanistan for detailed study regarding the sorption of insecticide in these blocks and the physical and chemical properties of the mud are as follows:—

Physical properties	Per cent
1. Specific gravity ...	2·56
2. Porosity	9·6
3. Permeability ...	
4. Sedimentation* ...	

Time, minutes	Per cent sedimentation
10	17
20	30
30	52
60	66
120	74
210	80
300	83

Chemical properties	Per cent
Calcium carbonate ...	7·25
Bicarbonate ...	0·0427
Total quantity of iron, phosphorus and aluminium oxides ...	13·22
Iron oxide (Fe_2O_3) ...	7·79
Phosphorus pentoxide (P_2O_5) ...	0·60
Aluminium oxide ($Al_2 O_3$) ...	4·83
Insoluble silica (SIO_2) ...	38·87

*The sedimentation was determined of the ground mud which was passed through a 16 mesh (ASTM) sieve. Thirteen per cent coarse material remained on 16 mesh sieve.

The mud blocks were sprayed with DDT suspension at the rate of 100 mg./sq. ft. and scrapings from 25 cm. area were removed 24 hours after spraying and once every week from the same block. The biological effectiveness of the deposits was also determined simultaneously. The quantity of DDT estimated from the scrapings and percentage mortality figures are given below:—

Age of deposit	DDT/mg. sq. ft.	BIOLOGICAL EFFECTIVENESS AGAINST *C. FATIGANS*			
		30 minutes Exposure		60 minutes Exposure	
		per cent mortality after 24 hours	after 48 hours	per cent mortality after 24 hours	after 48 hours
24 hours	64·4				
week					
1	53·28	...	...	...	...
2	30·5	...	...	...	...
3	4·5	82·5	100	92·5	100
4	28·44	79·5	93·7	96·5	100
5	4·5	65·0	86·7	93·7	95·0
6	16·92	48·7	67·5	77·5	85·0
7	15·12	25·0	35·0	38·75	62·0
8	10·8	12·5	22·5	25·0	40·0
9	10·6	7·5	17·5	...	...
10	8·58	2·5	12·5	...	...
11	0·294	0	0	...	...

There appears to be a close correlation between the amount of DDT present on the surface and the biological effectiveness of the deposit. Due to the presence of high iron-oxides content deposit of DDT 200 mg/sq. ft. was effective only for about eight weeks.

Micro-photographs from the same spot of the block were also taken every week. These definitely show the gradual disappearance of crystals after two weeks of application, but subsequently dust particles interfered with the results.

(*c*) *Measures to prevent sorption of insecticides into mud surfaces.*—As reported last year, glue and potassium dichromate mixture, glue and soap mixture and Ripolin clear coatings were tried to prevent sorption of insecticides into mud surfaces but none of these materials had been found satisfactory. In the light of further discussion with Technical Development Establishment Laboratory (who made the suggestion for the use of glue and potassium dichromate mixture) tests were repeated with slight modifications.

The modified formulæ used were as follows:—

1. Glue pot dichromate solution:
    Glue 6·0 parts.
    Potassium dichromate: 0·5 parts.
    Water: 93·5 parts.
2. Glue and soap solution:

Glue	...	...	1 lb.
Sunlight soap	...	...	½ cake.
Water	...	...	1 gallon.

Uniform mud blocks (6″ × 6″ × ½″) were treated with dichromate glue solution at the rate of 12·5 (750 mg.) and 25 c.c. (1,500 mg.) per sq. ft., and glue soap solution at the rate of 2 c.c. (250 mg.) and 4 c.c. (500 mg.) per sq. ft. The panels were dried for 48 hours and one set was then sprayed with DDT suspension at the rate of 50 mg./sq. ft., and another set with DDT emulsion supplied by the T.D.E.L. at the same rate. The test insects, *Culex fatigans* and *Musca nebulo*, were exposed to the treated surfaces for 15 minutes and were then kept for 24 hours under observation in clean cages. A new set of panels was used after every four weeks. These experiments are being continued as it was pointed out that mosquitoes at certain times of the year are more susceptible than at others. The data so far collected showed a slight increase in the duration of residual effectiveness but only by about a week or so.

5. *Efficacy of newer insecticides such as Malathion and Diazinon, etc.*

During the period under review the following newer insecticides of the phosphate series were tested for their biological effectiveness against houseflies and mosquitoes:

(*a*) *Malathion.*—$C_{10}H_{19}O_6$ PS_2, is the name given to the insecticidal chemical O—O—dimethyl— (1, 2— dicarboxy ethyl) dithiophosphate, belonging to phosphate series of compounds. It has been reported that unlike other compounds of the phosphate series it can be safely handled and is less hazardous.

A sample of Malathion in the form of 50 per cent emulsifiable concentrate was tested against houseflies *M. nebulo* and mosquitoes *C. fatigans* for its residual toxicity at dosages of 25, 50 and 100 mg. (Malathion) per sq. ft. on glass panels.

The test insects were exposed to the treated surfaces for 15 minutes and then kept in separate cages under observation for twenty-four hours after which the percentage mortality was recorded.

Malathion was found to be effective for 13 to 14 weeks against mosquitoes and 11 to 13 weeks against flies at the above dosages. Increase in dosage did not proportionately increase the duration of its effectiveness. DDT at 50 mg./sq. ft., when similarly tested, was effective for only five to seven weeks. The formulation, however, has a pungent odour which is rather unpleasant when used in a room.

(*b*) *Diazinon.*—Diazinon is an organic ester of thiophospheric acid (2-isopropyl-4-methyl-pyrimidyl-(6 diethyl ester). It has been reported to be effective against houseflies and mosquitoes, which have developed resistance to chlorinated hydrocarbon insecticides.

A sample of Diazinon, 40 per cent wettable powder, was tested for its residual toxicity at a dosage of 36 mg. Diazinon/sq. ft. (as advised by the firm) applied on glass panels in the same manner as described under Malathion. Diazinon was found to be effective for six weeks against mosquitoes and seven weeks against house-flies. DDT at 50 mg./sq. ft. tested similarly, was also effective for five and seven weeks, respectively. Diazinon has been reported to be comparatively more effective than chlorinated hydrocarbons as a larvicide. Further tests are in progress.

B. FIELD INVESTIGATIONS.

Comparative effectiveness of DDT, BHC, Dieldrin and Malathion residual sprays against mosquitoes.

The comparative effectiveness of the first three insecticides was studied in a number of villages in Madhya Bharat and Vindhya Pradesh in 1954 and preliminary results were reported last year at the Indian Council of Medical Research meetings. The final analysis of the data is briefly summarized below:

The results showed that out of the three types of DDT application (namely, uniform spray of houses with DDT 100 mg./sq. ft., spray of DDT (100 mg./sq. ft.) in the form of bands or strips (1½ ft. wide bands of untreated surfaces were left untreated between successive swaths) and spray of DDT 100 mg./sq. ft. to selective resting place of the vector species (the wall with the entrance door and opposite were left unsprayed). Best results were obtained with uniform application. DDT applied to the selective resting places was found to be more efficacious than that applied in the form of bands. Results on the effect of lime on DDT applications and the comparative efficacy of DDT and dieldrin were inconclusive.

Largescale trials with these four insecticides are being carried out this year in three different States, viz., Madhya Bharat, Vindhya Pradesh and Madhya Pradesh in collaboration with the malaria organizations in the respective States.

In each State, six areas have been selected and in each of the five areas one particular insecticide has been sprayed. The sixth area has been left untreated for purposes of comparison. Each area consists of a number of villages having a population varying from 3,000 to 10,000.

The following data are being collected from each of the six areas:

A. *Epidemiological:*

1. Pre- and post-spraying spleen and parasite rates in children (2-10 years old).

2. Infant parasite rate every month.

B. Entomological:

1. Comparative density of mosquitoes, anopheline and culicines and the survival rate of those caught in the sprayed areas every week.

2. Behaviour of vector species (*a*) daytime resting places, (*b*) time of entry into human dwellings for feeding and resting and the normal duration of each stay, (*c*) time and frequency of feeding.

Final assessment of the efficacy of each insecticide will be made after the malaria season sometime in December, 1955.

C. SAMPLES OF INSECTICIDES AND SPRAYING EQUIPMENT TESTED.

Insecticides		*Received from*
1. Strobane (Technical)	...	World Health Organization.
2. Pyrethrum extract	...	Messrs. Aikay & Co., Dehradun.
3. 50 per cent BHC water dispersible powder	...	Malaria Organization Coalfield supplied to them (Messrs. Bharat Pulverising Mills Ltd.).
4. 10 per cent shark liver oil	...	Government Shark Liver Oil Factory, Trivandrum.
5. Resitox	...	Dr. L. Kant, A.D.P.H., Malaria Control, Patna.
6. Hexamar BHC 50 per cent		Antimalaria Officer, Ganga Khadar, P.O. Hastinapur, Meerut.
7. DDT 50 per cent	...	,, ,, ,, ,,
8. Salvi mosquito repellent	...	Messrs. Salvi Medical Hall, Karnal.
9. BHC 50 per cent water dispersible powder	...	Messrs. Bharat Pulverising Mills Ltd.
10. Dialdrex	...	Messrs. Burmah Shell Oil Storage & Distributing Co. of India Ltd.
11. DDT (Technical)	...	Hindustan Insecticides Ltd.
12. Shalimar Insecticide Lacquer		Shalimar Paint Colour & Varnish Co., Delhi.
13. Thanite	...	Hardcastle Waud & Co. Ltd., Bombay.

Insecticides		*Received from*
14. 21 samples from Government Medical Stores Depot	...	Government Medical Store Depot, Madras.
15. Tossits	...	Burmah Shell Oil Storage & Distributing Co. of India Ltd.
16. DDT	...	R.H.P. & Co. through D.H.S., Orissa.
17. One chemical compound	...	Deptt. of Chemical Engineering, Delhi Polytechnic, Delhi.
18. DDT	...	The D.H.S., Tripura, Agartala.
Spraying equipment.		
1. Swing fog machine	...	Jardine & Henderson, New Delhi.
2. Stirrup pump sprayers	...	Messrs. Metal Alloy & Co., Calcutta.
3. Spray pump	...	Messrs. Kirloskar Bros., New Delhi.
4. Eclipse type of sprayers	...	Messrs. Gillanders Arbuthnot Co., New Delhi.
5. 7 hand compression sprayers	}	Received from various other sources.
6. 9 stirrup pumps		

2. Inquiry on the preparation of water-dispersible DDT under Dr. A. B. Biswas at the National Chemical Laboratory, Poona.

SYNOPSIS.

A number of water-dispersible DDT pastes containing between 45 and 80 per cent DDT and using imported wetting agents have been successfully prepared without the use of complicated or costly equipment. These pastes form surprisingly stable suspensions in water (suspensibility above 80 per cent), have greater covering power and persistency than any other available commercial dry powders. These pastes do not deteriorate on storage for one year at temperatures below 50°C. (122°F.).

Out of the wetting agents prepared from indigenous materials and tried in place of imported ones, saponin has been found to give very good results.

The preparation of dry wettable powders is being tried in three different ways; the work is in a preliminary stage.

Interfacial tension and contact angle (against DDT and BHC surfaces) of aqueous solutions of a large number of commercial wetting agents and those prepared in this laboratory, as also the sedimentation (volume and rate) of DDT in such media have been measured.

The phase studies of DDT and γ-HCH and their mixtures in various proportions have been made by the differential thermal analysis and the *x*-ray diffraction techniques.

INTRODUCTION.

DDT for insect control is generally used by spraying a solution or an emulsion of the material. Solutions and emulsions are expensive owing to the cost of the solvent. Water is the cheapest dispersion medium but

DDT, as it is, does not form a stable suspension because it is not wetted by water. Wetting and suspension stabilizing agents are, therefore, incorporated for getting a stable dispersion of DDT.

In a formulation, the size and shape of DDT crystals should be controlled properly to increase the covering power as much as possible. The reduction of the particle size by dry grinding is rather difficult as DDT forms very soft crystals which agglomerate by fusion during grinding. Hence, in the preparation of dry wettable powder in particular where the particle size has to be considerably reduced (below 20μ), one has to take the help of elaborate equipments, such as grinding mill, cyclone separator, etc. An inert clay material, such as talc is also added to the extent of about 25 per cent to help in the grinding process. The surface to volume ratio can be increased by changing the habit of the crystal from spherical to an acicular shape.

Because of the possibility that mixed crystals or solid solutions of DDT and another suitable insecticide will have more lethal power, phase studies of DDT, BHC systems have been made by the differential thermal analysis and the *x*-ray diffraction techniques.

PREPARATION OF CONCENTRATED PASTES AND THEIR STANDARDIZATION.

As reported last year, several water-dispersible paste formulations containing 45 to 80 per cent DDT have been evolved and their shelf-life, i.e., their stabilities with time and temperature tested. The pastes are reproducible and if kept below 50° C., their suspensibilities are little affected even after keeping for a period of one year. The advantages of these pastes over the commercial dry powders are the following:—

(*i*) These are easy to prepare and do not require the use of complicated or costly equipment.

(*ii*) These form surprisingly stable suspensions in water, the suspensibilities being of the order of 60 to 90 per cent as measured by a method specified by the W.H.O.

(*iii*) Their covering power is much greater due to the acicular shape of the DDT crystals, and being coated with a non-volatile lipoid soluble oil (waste lubricating oil), they are much more persistent. As a result the insecticidal efficiency is much greater than that of other commercial products. This has actually been found to be so, as per tests carried out by the Director of Public Health, Government of Bombay, Poona.

A possible disadvantage of the pastes may lie in the difficulties associated with their transport. Unlike a dry wettable powder, a paste cannot be transported in gunny bags. Metal containers would appear to be necessary for the purpose.

PREPARATION OF WETTING AGENTS FROM INDIGENOUS MATERIALS.

Next to DDT, the most important ingredient which largely determines the cost of formulation is the wetting agent. The following wetting agents were prepared from indigenous materials and their suitability was tested:—(1) Turkey red oil from castor oil, (2) sulfonated coco-nut oil,

(3) potassium and ammonium oleates, (4) potassium abietate, (5) abietene sulfonic acid (4 and 5 have been obtained from turpentine rosin, a cheap by-product available from turpentine distilleries), and (6) saponin from soapnuts. Except saponin, none others have given satisfactory results so far. Pastes containing saponin have given quite stable suspensions the suspensibilities being above 80 per cent. The standardization of the process is in progress.

MEASUREMENT OF INTERFACIAL TENSION, CONTACT ANGLE AND SEDIMENTATION (VOLUME AND RATE).

Since a large number of wetting agents had to be tried in these formulations, it was necessary to adopt simple and quick physical methods which would indicate their suitability with regard to wettability, adhesion and decreasing the attraction between dispersed particles. Interfacial tension, contact angle and sedimentation (rate and volume) measurements supply such information. The necessary apparatus for making such measurements was improvised.

Surface tension.—The surface tension of a large number of commercial wetting agents and of those prepared at the N.C.L. was measured in aqueous solutions at 30° ± 0·2° over a range of concentration of 0 to 1 per cent by the ring balance as well as Sugden's maximum bubble-pressure method. The results are summarized in Table I.

Contact angle measurement.—The wettability and the adhesion tension between a solid and a liquid were estimated by measurements of contact angles by the tilting plate method. This instrument was designed and constructed by fixing a transparent protractor graduated to read ±0·5°, on a circular brass plate which could be rotated by means of a bakelite knob. A microscope slide was fixed to a holder attached to the brass disc and the angle read directly. Glass slides were coated by spraying concentrated solutions of DDT or BHC in benzene. A transparent rectangular trough 4″ × 1″ × 1″ was filled with the aqueous solution of a wetting agent and the half-immersed coated slide was tilted slowly until the surface of the liquid remained undistorted right upto the line of contact with the plate. The trough was illuminated and the surface was observed through a travelling microscope. The results are given in Table II.

Sedimentation (volume and rate) measurement.—A wetting agent also decreases the attraction between the dispersed particles; the effect has been estimated by sedimentation (rate and volume) measurements. For this a known weight of DDT is thoroughly mixed with the aqueous solution of the wetting agent in a graduated cylinder and the volume of the mass sedimented is noted at definite time intervals. The smaller the sedimentation volume, the more effective is the surface active agent as a suspending agent. The results are given in Table III.

TABLE I.

Surface tensions of surface active agents in aqueous media at 30°C.

Per cent concentration w/v	Lissapol NX	Triton X-100	Nonex 39	Tergitol 4	Antaron L-135	Antaron N-185	Antaron R-275	Nansa HS	Petroleum sulphonate	Triton 770	Sodium lauryl sulphate	Amine 220	Saponin	Potassium abietate	Potassium oleate
0·0000	71·5	71·5	71·5	71·5	71·5	71·5	71·5	71·5	71·5	71·5	71·5	71·5	71·5	71·5	71·5
0·0001	61·0	61·8	...	...	...	...	...	...	...	...	...	62·6	...	...	...
0·00025	53·5	...	64·8	...	66·0	...	...	...	...	61·0	64·0	56·3	...	...	...
0·0005	47·0	53·7	...	...	...	...	...	64·5	...	54·0	...	47·0	...	...	...
0·001	43·0	48·5	56·6	...	61·7	65·1	71·0	62·7	71·0	51·0	60·4	44·0	71·0	68·3	65·1
0·002	37·0	...	...	...	58·7	...	64·9	62·7	...	47·5	...	37·0	...	64·8	...
0·005	33·0	35·5	43·0	...	46·3	51·8	...	56·8	61·9	40·0	...	...	60·5	60·7	53·6
0·010	31·0	31·6	39·0	62·0	45·2	37·5	52·6	50·0	53·7	35·0	40·6	29·7	52·6	56·2	49·5
0·025	30·6	31·0	34·0	57·0	34·0	29·6	49·0	45·0	46·0	29·0	37·0	28·8	50·5	52·0	27·7
0·050	30·3	31·0	33·3	50·8	30·7	29·5	44·4	38·0	41·4	28·0	34·9	28·4	49·2	49·2	25·2
0·10	30·0	30·4	31·6	46·9	31·7	28·0	37·1	32·0	39·9	27·7	34·9	28·4	43·2	46·1	25·2
0·20	...	...	...	43·5	...	...	...	...	...	...	34·9	...	...	44·0	...
0·50	...	...	...	35·7	...	...	...	...	...	...	36·6	...	41·6	...	...
1·00	30·0	30·4	31·6	30·1	30·9	27·4	30·4	28·0	35·5	...	36·6	28·4	41·4	37·1	25·2

TABLE II.

Contact angles (DDT—wetting agents in aqueous media) at room temperature.

Per cent concentration w/v	Lissapol NX	Triton X-100	Tergitol TMN 650	Nonex 39	Triton 770	Teepol 610	Antaron L-135	Antaron N-185	Antaron R-275	Lissapol LS	Nansa HS	Petroleum sulphonate	Amine 220	Lauryl pyridinium chloride	Saponin	Turkey red oil	Sodium lauryl sulphate	Potassium abietate
0·0000	108	108	108	108	108	108	108	108	108	108	108	108	108	108	108	108	108	108
0·0005	45	57	75	66	95	97	60	50	...	76	...	...	58	...	...	...	...	...
0·001	25	50	58	48	85	89	43	36	...	56	...	101	31	...	92	...	72	...
0·002	10	26	40	33	70	76	27	16	...	...	93	68	19	...	78	...	63	...
0·005	2	15	22	16	41	53	15	8	100	34	79	36	8	88	70	73	54	82
0·010	0	8	14	8	21	40	10	0	85	17	71	22	0	72	60	61	27	71
0·020	...	...	...	...	8	...	...	...	70	...	56	8	...	55	48	52	...	60
0·040	...	...	...	...	...	...	...	...	53	...	46	...	...	...	...	...	...	53
0·050	...	...	...	...	...	...	...	...	...	...	...	...	...	39	37	...	...	...
0·060	...	...	...	...	...	...	...	...	37	...	...	...	...	...	...	36	...	49
0·080	...	...	...	...	...	...	...	...	26	...	...	...	...	...	...	26	...	...
0·100	...	...	...	...	...	...	...	...	18	...	8	...	...	8	20	...	...	20
0·200	...	...	...	...	...	...	...	...	8	...	...	...	...	0	0	...	...	...

TABLE III.

Sedimentation volumes of DDT in various wetting agents at room temperature.

Wetting agent	Per cent concentration w/v	SEDIMENTATION VOLUME IN C.C.			
		1 minute	2 minutes	5 minutes	24 hours
Lissapol NX	1·00	3·45	3·70	3·75	3·80
	0·20	4·85	4·80	4·80	4·80
Triton X-100 ...	1·00	3·30	...	3·50	3·60
	0·20	4·65	4·60	4·60	4·60
Tergitol TMN 650 ...	1·00	4·31	4·30	4·30	4·30
	0·20	5·10	5·10	5·10	5·00
Nonex 39	1·00	4·40	4·40	4·40	4·40
	0·20	5·30	5·30	5·30	5·25
Teepol 610	1·00	5·00	5·00	5·00	...
	0·20	5·25	5·20	5·20	...
Antaron L-135 ...	1·00	4·80	4·80	4·80	...
	0·20	5·00	5·00	5·00	...
Antaron N-185 ...	1·00	3·65	3·79	3·82	3·81
	0·20	3·80	3·82	3·90	3·85
Antaron R-275 ...	1·00	4·85	4·80	4·75	4·60
	0·50	5·10	5·00	4·90	4·65
Nansa HS	1·00	4·38	4·39	4·36	4·30
	0·20	4·75	4·72	4·70	4·60
Petroleum sulphonate ...	1·00	4·68	4·69	4·68	...
	0·20	5·10	5·09	5·07	...
Amine 220	1·00	4·40	4·35	4·30	4·20
	0·20	4·50	4·30	4·30	4·20
Saponin	1·00	4·20	4·25	4·30	...
	0·20	4·20	4·25	4·30	...
Turkey red oil	1·00	5·00	5·00	5·00	...
	0·50	5·10	5·10	5·10	...
Sodium lauryl sulphate ...	1·00	4·90	4·90	4·89	4·78
	0·20	5·00	4·97	4·95	4·85
Potassium abietate ...	1·00	4·35	4·38	4·38	4·38
	0·30	5·20	5·20	5·20	5·10
Potassium oleate ...	1·00	4·15	4·20	4·20	4·19
	0·20	4·47	4·45	4·41	4·40

PREPARATION OF DRY WETTABLE POWDERS.

This work is in a preliminary stage. The preparation of the wettable powder requires efficient grinding and the dispersion of the product in a very fine powder form. For this purpose, it is proposed to study the relative merits of the following three likely processes:

(*i*) Preparing a concentrated aqueous paste by wet grinding and spray-drying the product.

(*ii*) Dry grinding of DDT together with wetting and dispersing agents and inert diluents.

(*iii*) Mixing DDT, wetting agent, etc., in a molten condition and atomizing the molten mass by allowing it to pass through a spray gun type of device while controlling air and material feed in such a way as to get particles of the right order of size in a single pass. The problem of efficiently collecting the product will have to be considered. If this process could be successfully worked out, it would be the easiest and cheapest method of preparation of wettable dry powders.

A few trial experiments have been made. The results are, however, inconclusive. A more thorough study, particularly of the design of the apparatus is required.

PREPARATION OF PASTES CONTAINING MIXTURES OF INSECTICIDES.

The possibility of mixed crystal or solid solution formation between the active isomers of pp′ DDT and γ-HCH in various proportions has been investigated by the following methods: (*i*) differential thermal analysis, (*ii*) *x*-ray diffraction, and (*iii*) density measurements of the mixtures. The data are being processed.

Water-dispersible pastes containing mixtures of technical DDT and technical BHC have been prepared and sent for biological assay to (*i*) The Director, Public Health Department, Government of Bombay, Poona, and (*ii*) The Direcor, Malaria Institute, Delhi. Their results are awaited.

DISCUSSION AND CONCLUSION.

The contact angle gives a measure of the adhesion between the solid and the liquid and surface tension measures the spreading power; lower values in both cases indicate better adhesion and spreading power. From the data in Tables I and II, it is evident that Lissapol NX is the most suitable wetting agent for making stable DDT suspensions. Amine 220, triton X-100 and antaron N-185 are also nearly as good as lissapol NX.

Sedimentation (volume and rate) data give an idea of the degree of deflocculation, lower values again indicating better dispersion in the medium. From the data in Table III it is inferred that lissapol NX, triton X-100, potassium oleate, antaron N-185 and saponin are good dispersing agents for DDT.

Paste compositions containing 50 to 70 per cent DDT were made on a one-half lb. scale incorporating wetting agents and their suspensibilities were measured. The results were in general agreement with those suggested by surface tension, contact angle and sedimentation data. Saponin was found to yield pastes having unexpectedly high suspensibilities. This may be due to the pectinous substances present in saponin, which act as effective suspension stabilization agents.

It was noticed that most of the wetting agents suitable for the present purpose such as lissapol NX, triton X-100 and saponin are of the non-ionic type, which are synthetic products with the exception of saponin.

In the preparation of dry wettable DDT powder, it is desirable to use wetting agents which are solid at room temperature and which are non-hygroscopic. It may be worthwhile to investigate the usefulness of antaron N-185 and potassium oleate for such purposes.

3. Inquiry on the factors influencing the development of malaria parasite in the mosquito by cytochemical technique under Professor H. N. Ray at the School of Tropical Medicine, Calcutta.

During the period under review, histochemical techniques were employed to determine the chemical make-up of the sporogony cycle of the parasite *Plasmodium gallinaceum* and its environmental tissue with a view to ascertain the chemical adaptation of the parasite to its environment.

For the purpose of these experiments *Ædes ægypti* mosquitoes bred in this laboratory were used as vectors. The cytochemical techniques employed were as follows:—

Toluidine blue (Brachet), pyronin-methyl green (Brachet), Feulgen's nucleal reaction, PAS-technique (Hotchkiss), acetic iron-ferrocyanide test for hyaluronic acid type of polysaccharides (Hale) and Gomori's revised technique for alkaline phosphatase.

Cytochemical make-up of the parasite.—The ookinete contained very little of ribonucleic acid as suggested by the very weak stain with pyronin. In the nucleus of ookinete the reaction for desoxyribonucleic acid was, either, entirely absent, or there was a very weak and diffuse reaction. Toluidine blue stained the ookinetes very weakly and no metachromasia could be detected. Very weak reaction for alkaline phosphatase was detected in the nucleus, while the cytoplasm was free of this enzyme. Along the periphery of the cell-body there appeared a series of minute granules which showed a faint positive reaction for alkaline phosphatase. PAS test for polysaccharides yielded negative result.

It was, however, interesting to note that the ookinetes which had just pierced the gut-wall and had come to lie projected into the hæmocoelic cavity developed a distinct PAS-positive border, and also showed positive reaction for alkaline phosphatase. In such forms the reaction for DNA in the nucleus also seemed stronger.

The absence of any reserve food-storage in the ookinetes of *Plasmodium gallinaceum* is sharply at variance with what was seen in the homologous forms, i.e., the oocysts of the *Coccidium eimeria tenella* (Gill and Ray, 1954, *Ind. Jour. Vet. Sci. & Anim. Husb.*, **24,** p. 223). The difference, it seems, is correlated to the different modes of life of the two parasites. The ookinetes when inside the stomach can directly receive nourishment from blood or the products of blood. But when they come to lie suspended into the hæmocoelic cavity, a new source of food, viz., the hæmocoelic fluid is apparently drawn upon. It seems that the PAS-positive border of the late ookinete, which is also positive for alkaline phosphatase reaction is associated with phosphorylation and dephosphorylation processes at that site. The increase of DNA in the parasite from this time onwards marks a great change in its physiology.

In the oocysts, mucopolysaccharides could be detected with toluidine blue stain. These appeared in the form of metachromatic granules, lying scattered in the cytoplasm of the developing oocyst on the 4th day after feed. These were found in the neighbourhood of the hæmozoin pigment, or even associated with them. The large number of nuclei in the developing oocyst took up deep stain, while the cytoplasm appeared light blue. The nuclei of such oocysts reacted positively with Feulgen's nucleal reaction and were also positive to the hyaluronic-acid type of polysaccharide. The reaction for alkaline phosphatase was found to be very strong in the oocysts. The nuclei showed heavy deposition of cobalt sulphide, while the cytoplasm appeared light grey.

The above observations constitute a close parallel to what was observed in the *Coccidium eimeria tenella*, (*loc. cit.*), so far as the significance of the mucopolysaccharides in the life-cycle of the parasite is concerned. Mucoid sulphates are found to occur in the cytoplasm, while the hyluronic-acid type of polysaccharide is found to occur in the nucleus. It seems that by virtue of their hydrotropic properties they prevent intracytoplasmic clotting and create a physiological liquefaction of the nucleus during sporozoite formation.

According to Lewert (*Jour. Inf. Dis.*, 1952, **91.** p. 125), the salivary gland preparations of the mosquito infected with *P. vivax*, uninucleate or quadrinucleate forms of sporozoite were observed, but none of the nuclei were Feulgen-positive. In course of the present investigations, however, uninucleate sporozoites have been obtained in the mature oocysts, as well as in the salivary glands. The nucleus of the sporozoites consisted of a compact mass of DNA, situated near the middle of the cell-body. Sometimes a DNA containing central karyosome could also be detected. Similar distribution of DNA was also detected with methyl-green stain, while the cytoplasm was very faintly pyronin-positive. Strong reaction for alkaline phosphatase was detected in the nucleus of sporozoites. This enzyme was also present in the cytoplasm though in a lesser amount.

Histochemical make-up of the environmental tissue of the parasite.—The general interest is centred round the stomach of the mosquitoes where the parasite undergoes its early stages of development. Information is, however, scanty as regards the changing conditions which constitute the environment of the ingested parasite, met with in the stomach, during and after the digestion of blood. Pal (1943, *Jour. Mal. Inst. India*, **5,** p. 247) in course of his histological observations on the stomach of a number of species of mosquitoes (both *anopheles* and *culex*) noted that the stomach epithelium varied greatly according to the state of the digestive processes, but the details of such variations were not discussed by him. In course of the present investigation following observations have been made on the stomach wall of the vector mosquito:—

Stomach wall when blood is not ingested.—The epithelial cells were characterized by a large nucleus, rich in DNA, while the cytoplasm was voluminous and lodged a prominent globule of RNA-containing material towards the lumen-end of the cell. RNA-material was also noticed adhering to the outer surface of the nuclear membrane. It seemed possible that the latter coalesced to form the RNA globule referred to above.

In a section of the stomach these cytoplasmic inclusions in the form of globules could be seen arranged in a row, one in each cell. These are indicated as 'ergastoplasm' which is now recognized as a basophilic or chromophilic component of the cytoplasm and in characterized by certain properties, such as a high RNA-content.

Stomach wall during digestion of blood.—Following blood feed in the stomach epithelium showed intense activity. This involved discharge of parts of cells, loaded with granular matter and containing the 'ergastoplasm', inside the lumen. Such parts of cells also sometimes took with them the nucleus. The cellular substance thus discharged in the lumen of the stomach could be found lying scattered in the midst of the ingested blood even 24 hours after feed. These appeared to disintegrate later.

4. Biochemical investigations in malaria with particular reference to protein metabolism of *P. gallinaceum* in chick malaria under Dr. M. Sirsi at the Indian Institute of Science, Bangalore.

Arrangements to carry on large-scale hatching of eggs were made by bringing another incubator into commission. A fresh strain of *P. gallinaceum* required for the investigations was obtained from the Southern Branch of Malaria Institute, Coonoor, and is being maintained in chicken by weekly transfers.

Chicken, four weeks old, and of the white leghorn breed were chosen for the experiment. They were housed in individual cages and kept on standard laboratory diet. Blood samples were usually taken in morning by heart puncture and by using sodium citrate as the anti-coagulant. 0·1 c.c. samples of blood were pippetted out into 50 c.c. conical flasks and autoclaved with 25 c.c. of 6 N hydrochloric acid (for acid hydrolysis) and 500 mg. of barium hydroxide (selected after some preliminary experiments) for alkaline hydrolysis. An equal volume of blood taken from the same sample was extracted with 5 c.c. of 75 per cent alcohol and the residue got after centrifugation at high speed was quantitatively transferred to another conical flask and subjected to acid hydrolysis. A definite volume of the alcoholic extract was also used for amino-acid analysis. Acid and alkaline hydrolysis were conducted for six hours at 15-lb. pressure after which they were quantitatively filtered, evaporated on water bath to remove the acid vapours and taken in 5 c.c. of distilled water in a neutralized condition suitable for chromatographic analysis. In the case of alkaline hydrolysates excess of barium hydroxide was removed with dilute sulphuric acid and the filtrate was evaporated and taken in 5 c.c. of water for subsequent analysis.

The same groups of birds were infected intramuscularly with 16 million parasites and blood samples were taken during incubation period and at the time of high parasitæmia. These blood samples were also subjected to the above treatment before starting with the chromatographic anlaysis for the estimation of the amino acids.

Amino-acid analysis of these hydrolysates was carried out by the paper chromatographic technique as reported by Rao *et al.* (*Jour. Ind. Inst. Sci.*, 1955, **37,** p. 130). For each hydrolysate three chromatograms developed with three different solvent mixtures have been used in the procedure and the chromatograms have been developed in standard trays at 60°F to 64°F. with previously washed (N/100-HCl and distilled water) Whatman No. 1 filter papers to get quantitative values for individual amino acids. After developing these chromatograms thrice they were air-dried and sprayed with four per cent ninhydrin in 95 per cent acetone and heated at 65° for 15 minutes. The coloured bands, so got, were first identified in preliminary experiments and then various bands were cut out, extracted with 5 c.c. of 75 per cent alcohol saturated with copper sulphate (Giri *et al.*, *Jour. Ind. Inst. Sci.*, 1953, **35,** p. 145) and the colour intensities were compared in a Klett Summerson's Colorimeter using Filter No. 54. From the values got with standard solutions of the amino acids the exact amount of the amino acids in the blood samples were calculated.

The results so far obtained indicate that the amino-acids leucine, iso-leucine, phenylalanine, alanine, arginine, glutamic acid and threonine are increased during the incubation period and they are decreased in the parasitized condition, the decrease being more specific in the case of a few amino acids-like leucine and threonine. In the case of the analysis of the alcoholic extract (75 per cent) a new band has been observed in the position of cystine and further work is in progress to elucidate its nature. The above investigations are being done with reflex hydrolysates also to confirm the above findings as suggested by the reports of Williams (*Cornell University Memoir*, **337,** 1955). Amino acid analyses of the plasma and erythrocytes are also in progress as per the plan submitted for the previous year.

5. Inquiry on the study of the life-history, bionomics, toxonomy and disease transmitting capacity of members of family Ceratopogonidæ under Dr. B. C. Basu at the School of Tropical Medicine, Clacutta.

The literature on the family ceratopogonidæ is scattered and, as far as consulted, it is mostly in English and some are in French and German. Account of a large number of genera with many species in some of them has been noted. Of the five blood-sucking genera, viz., acanthoconops, holoconops, leptoconops, culicoides and lashiohelea, culicoides has a fairly world-wide distribution with a record of 28 species in India and 97 from Africa, America, West-Indies, Burma, Ceylon, Europe and Malaya, while the other four genera have a very limited distribution with only a few species under each genus. The same species and genus described by the different workers under different names is of common occurrence in the literature and in many cases it is imperfect. Many authors have referred to a group of species or genera and have devised keys for them without any consideration for the rest. Description of new species without any reference to previous and allied work is not uncommon. Descriptions of 405 species under all the genera so far studied from the literature are being examined for regularizing them in a handy key form after eliminating the incomplete, incorrect and synonymous ones.

Regular field collection of adults of this family is being made from cattle-sheds in the fringe area of Calcutta as well as by setting up a light trap in Calcutta city. By both these ways a good collection of adults has been made. In cattle-sheds, *Culicoides anopheles* are frequently collected and found parasitizing *Anopheles barbirostris*. Besides these specimens from the Zoological Survey of India have been received for taxonomic work. Some adults were reared from the larvæ collected in the field.

In the field, a good collection of larvæ and pupæ could be obtained from:—

(1) Clear water at the margin of ponds.
(2) From shrubs collected from ponds.
(3) From rotten material at the bases of banana plants.

It is found that the habitats of the larvæ are aquatic, semiaquatic and terrestrial. It seems that, in nature, they oviposit in the sites from which the larvæ are collected. But so far samples of eggs could not be collected from those sites. Some of the larvæ could be reared in the laboratory. Egg laying of a wild adult culicoides was noted under laboratory conditions in a test-tube. It laid eggs in batches of from 1 to 30 totalling 199. When freshly laid, an egg was milky white gradually changing its colour to dark brown. It has been noticed that the larvæ collected from the field pupate in 4 to 12 days' time and the pupal stage lasted for two to eight days. A few media have been tried to rear the larvæ collected from the field but so far pond water with some banana bark crushings was found to be best suited. Material of egg, larva, pupa and adult has been prepared for morphological studies.

NUTRITION.

1. Nutrition Research Laboratories, Coonoor.

I. STUDIES ON VITAMINS.

Vitamin A.

A fluorometric method for vitamin A assay.—It has been known for some time that vitamin A gives green fluorescence under ultraviolet light and that continuous irradiation causes rapid and complete destruction of the vitamin. The alcohol and the acetate forms of vitamin A differ slightly in this respect; the latter on irradiation shows initially a steep increase in fluorescence followed by quick deterioration. A method is described below for the quantitative estimation of vitamin A taking advantage of its fluoresceing property.

The material containing vitamin A was saponified with alcoholic potash under reflux, using all-glass apparatus. The unsaponifiable fraction was extracted three or four times with glass distilled low boiling petroleum ether, washed repeatedly with distilled water to remove alcohol and soap, treated with pure anhydrous sodium sulphate to remove moisture, filtered and made to volume. Aliquots were used for estimation of vitamin A.

For fluorometric assay, 10 c.c. portions of the extracts (or an aliquot diluted to 10 c.c.) were used. The source of ultraviolet light was a mercury arc lamp of 1,000 watts placed at a distance of about 10 cm. from the cuvette. The exciting wavelength was 365 $m\mu$. selected by filtering the initial light through Corning filter 5874. A combined filter, Corning 3389 and 4308, was interposed between the cuvette and the photometer to eliminate the interfering wavelengths. The photometer used was a Photovolt Multiplier Photometer, Model 520 M, which is an extremely sensitive instrument and is capable of reading very feeble light values down to 10^{-4} microlumen. The fluorescence values were read from the deflections on the transmission scale of the galvanometer of the instrument. The difference between the readings obtained with the reagent blank and the unknown solution, respectively, was taken as a measure of the fluorescence due to vitamin A in the solution. For the calculation of vitamin A values, galvanometer readings of the unknown solutions were compared with those of the known standards of approximately similar strengths. A standard curve was also prepared from solutions of pure vitamin A. A linear relationship between fluorescence and the concentration of vitamin A, for the range 1 to 18 I.U. per 10 c.c. was found. At higher concentrations this relationship did not hold good.

The use of all-glass apparatus and glass distilled pure solvents was necessary to eliminate irrelevant fluorescence. It was observed that petroleum-ether solutions of ghee, vitaminized vanaspati (hydrogenated fat) and common vegetable oils, e.g., groundnut, coconut and sesame etc., show considerable irrelevant fluorescence. Saponification of these materials helped to reduce the fluorescence by about 50 to 70 per cent. The irrelevant fluorescence still present in the solutions containing the non-saponifiable material would correspond to about 12 to 24 I.U. vitamin A per gramme of fat. Ultraviolet irradiation of the solutions of non-saponifiable matter from different fats for up to 2½ hours did not alter the irrelevant fluorescence of groundnut oil, coconut oil and sesame oil, while that of ghee and vanaspati was reduced to a constant value. In latter cases the difference in fluorescence before and after irradiation was taken as the measure of vitamin A content.

The values of vitamin A determined by the fluorometric method and those obtained by spectrophotometric or colorimetric methods for a few natural sources of vitamin A were found to be in good agreement.

Fluorescence property of associated compounds.—Since vitamin A in nature may be associated with unsaponifiable substances, such as vitamins D and E, cholesterol and carotene, it was considered worthwhile to study their influence on fluorometric assay of vitamin A. Under the experimental conditions described above, vitamin D (25 μg. calciferol) and carotene (100 μg. of 90 per cent β-carotene) did not fluoresce; while vitamin E (α-tocopherol acetate) and cholesterol showed very feeble fluorescence; as much as 5 mg. of these substances were required to give fluorescence equal to that of 1 I.U. of vitamin A. Ultraviolet irradiation did not alter the fluorescence property of vitamin D or cholesterol while that of vitamin E was completely destroyed.

VITAMIN D.

Metabolism of rachitic epiphyseal cartilage and the effect of vitamin D.—The adverse effect of vitamin D deficiency on the capacity of rachitic rat cartilage to oxidize pyruvic acid which was reported last year has formed the starting point for a further series of investigations on the mode of vitamin D action.

Pyruvic acid is the precursor of the acetyl group which on condensation with oxalo-acetic acid gives rise to citric acid, an important intermediate in the Krebs tricarboxylic acid cycle of carbohydrate metabolism. A diminution or loss of the capacity to oxidize pyruvic acid would, therefore, be expected to lead to a diminished formation of citric acid. Apart from its rôle in carbohydrate metabolism, citric acid is of direct importance in bone metabolism as is shown by the fact that (a) bone salt contains citrate, (b) citrate-citric acid mixture brings about healing of induced rickets (Shohl, *Jour. Nutr.*, **14,** 69, 1937) and (c) level of citrate in the serum of rachitic infants is lower than in normal infants (Harrison and Harrison, *Nutr. Rev.*, **10,** 233, 1952).

Studies were, therefore, undertaken to determine the effect of vitamin D deficiency in rats on (a) citric acid content, and (b) the citrogenase activity of the rachitic epiphyseal cartilage. The technique of inducing rickets and studying the effect of a single large dose of vitamin D has been described by Tulpule and Patwardhan (*Biochem. Jour.*, **58,** 61, 1954). Estimations of citric acid were carried out in epiphyseal cartilage of rachitic and vitamin D treated rats. The results showed a 100 per cent increase in cartilage-citrate within 24 hours after the administration of vitamin D. Preliminary experiments have shown that the increase in citric acid in cartilage reached a maximum at 96 hours after vitamin D feeding.

The method of Dixon and Perkins (*Biochem. Jour.*, **52,** 260, 1952) was used to determine the citrogenase activity of rat cartilage in rachitic and vitamin D treated animals. It appeared that rachitic cartilage had some activity, for a small amount of citric acid was being formed from the reaction mixture of acetate and oxaloacetate by cartilage slices. When tested after 96 hours subsequent to vitamin D administration there was a 100 per cent increase in citrogenase activity as expressed in terms of citric acid formed per gramme of fresh tissue in one hour at 30° C.

Confirmation of these results is being sought in a fresh series of experiments with a larger number of rats and also in a different species of animals such as dogs. It would be premature to build up an elaborate hypothesis on the findings reported above ; it is gratifying to note, however, that the current findings fit in with those made earlier on pyruvate oxidation in rachitic cartilage.

II. STUDIES ON PROTEINS

1. Essential amino acids in some vegetable foodstuffs.

Balasubramaniam *et al.* (*Ind. Jour. Med. Res.*, **40,** 73 and 219, 1952) have determined by microbiological techniques the essential amino acid composition of some cereals commonly consumed in India. Vijayaraghavan and Srinivasan (*Jour. Nutr.*, **51,** 261, 1953) have subsequently determined the essential amino acids in common Indian pulses. In addition to the foodstuffs analysed by the above workers, there remain quite a few cereals, legumes and several tubers which find a place in Indian dietaries. They contribute appreciable amounts of protein in the diet; hence it was considered worthwhile to determine their essential amino acid composition. All the amino acids were assayed by microbiological methods. The average values are given in Table I.

It will be seen from the Table I that although cereal proteins, in general, are poorer in lysine than the whole egg protein, a certain species of amaranthus known as ' Rajkeera ' compares well with the whole egg in this respect.

Cereals, legumes and tubers are deficient in methionine, the deficiency being the greatest in the latter two. Deficiency in tryptophane was also present although it was less marked than that of methionine. Legume proteins in general have a high lysine content and thus can supplement cereals in Indian diets with some advantage.

During the course of this investigation certain variations in the amino acid content of proteins in different varieties of the same foodstuff were observed. For example, in the case of *kodra* and sweet potato the strains with higher protein content had less essential amino acids per 16 g. N than the strains with lower protein content. This observation is in line with those of Mitchell *et al.* (*Jour. Nutr.*, **48,** 461, 1952) and Sauberlich *et al.* (*Jour. Nutr.*, **51,** 241, 1953) on proteins of maize.

2. Duck egg white (DEW) ovomucoid.

Considerable evidence has been forthcoming (Narasinga Rao, and Patwardhan (*Ind. Jour. Med. Res.*, **42,** 533, 543, 1954) to show that the low nutritive value of duck egg white (DEW) is primarily due to an ovomucoid contained in it which has antitryptic and growth retarding properties. Further work on the biological action and properties of the ovomucoid is described in the following paragraphs.

TABLE I.

Essential amino acid content of some vegetable foodstuffs.

(Values expressed in g. per 16 g. of total nitrogen).

Name	Botanical equivalent	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl-alanine	Threonine	Tryptophan	Valine
I. *Cereals*											
Kodra	*Paspalum scrobiculatum*	4·0	1·8	5·3	8·5	2·1	2·8	6·9	3·8	0·6	5·6
Rala	*Setaria italica*	3·6	2·1	7·6	16·7	2·1	2·8	6·7	3·1	1·0	6·9
Samai	*Panicum milliare*	4·7	1·9	6·7	10·9	1·8	2·3	4·8	3·4	0·6	6·1
Rajkeera	*Amaranthus paniculatus*	14·8	2·9	6·9	8·0	8·2	2·5	4·8	4·4	0·9	6·1
II. *Legumes*											
Horse gram	*Dolichos biflorus*	5·4	3·0	6·7	7·9	8·3	0·8	8·5	3·8	0·6	5·4
Khesari dal	*Lathyrus sativus*	7·8	2·5	6·6	6·6	6·9	0·4	4·1	2·3	0·4	4·7
Wal	*Dolichos lablab*	9·2	2·8	6·0	8·9	8·1	0·7	5·3	3·3	0·5	5·6
Peas	*Pisum sativum*	17·7	2·7	8·4	10·9	11·4	1·3	5·8	4·9	0·7	7·8
III. *Tubers and others*											
Sweet potato	*Ipomœa batatas*	5·2	1·5	6·2	6·8	5·0	1·4	5·0	5·3	0·8	9·5
Elephant yam	*Amorphophallus campanulatus*	11·2	1·6	5·0	5·9	4·4	1·0	6·2	4·5	0·6	4·9
Colocasia	*Colocasia antiquoram*	6·2	1·7	5·2	8·9	6·2	1·0	5·2	4·7	1·4	6·0
Potato	*Solanum tuberosum*	5·2	1·2	5·3	5·4	6·7	1·0	4·1	3·9	0·7	5·0
Tapioca	*Manihot utilissima*	7·7	1·5	5·3	5·6	6·2	0·6	3·4	3·8	0·5	4·5
Green plantain	*Musa paradisiaca*	4·1	4·5	5·1	5·4	5·6	0·6	4·5	2·7	0·7	4·3

Effect on growth of varying levels of protein intake in presence of ovomucoid.—It has been demonstrated (*Ann. Rep.*, 1953-54, p. 2) that the ovomucoid exerted the maximum inhibitory effect at two per cent level of intake. Further experiments were undertaken to determine the effect of varying levels of protein in diet at a fixed level of ovomucoid. For this purpose six groups of rats were fed diets containing casein at 5, 10 and 20 per cent levels without and with DEW ovomucoid at one per cent level. The results summarized in Table II will show that at higher protein levels the inhibitory effect of ovomucoid is fully overcome.

TABLE II.

Effect of varying protein levels on growth-retarding effect of DEW ovomucoid.
(Six rats in each group).

Diet containing	AVERAGE WEIGHTS		
	Initial, g.	Final, g.	Gain in 4 weeks, g.
Five per cent casein— Control ...	53·3	86·3	33·0 ± 1·71
,, ,, + 1·0 % DEW ovomucoid ...	53·0	62·5	9·5 ± 2·96
Ten per cent casein— Control ...	53·3	108·2	54·9 ± 1·83
,, ,, + 1·0 % DEW ovomucoid ...	53·3	85·0	31·7 ± 2·15
Twenty per cent casein–Control ...	53·2	119·8	66·6 ± 3·22
,, ,, + 1·0 % DEW ovomucoid ...	53·2	111·5	58·3 ± 1·82

Effect of intraperitoneal administration of ovomucoid.—The finding that DEW ovomucoid depressed growth even when fed with casein hydrolysate necessitated investigating whether the former had a systemic action in addition to its antitryptic effect which is normally exerted in the intestines. It was possible that ovomucoid which has a smaller molecular size compared with many other proteins could be absorbed intact from the intestines in small quantities and thus be in a position to exert a systemic effect. Three groups of rats were maintained on a ten per cent casein diet. To animals of two groups, daily intraperitoneal injections of 1 c.c. of a five per cent saline solution of purified HEW and DEW ovomucoids, respectively, were given for 28 days. The animals in the control groups received injections of physiological saline solution. The average growth of rats in all the three groups was very nearly the same.

The above results clearly demonstrate that the ovomucoids had no systemic action even in fairly high doses. One can reasonably conclude, therefore, that the site of the inhibitory action which DEW ovomucoid exhibits when fed with casein hydrolysate is also probably the intestine.

The effect of aureomycin on growth retardation by DEW ovomucoid.—When aureomycin was included in a diet containing ten per cent unautoclaved duck egg white, considerable improvement in growth of rats (*Ann. Rep.*, 1953-54, p. 4) was obtained but no such effect was noticed with autoclaved DEW and aureomycin. A study of the digestibility of the unautoclaved DEW protein with and without aureomycin failed to reveal any significant differences. Hence, it appears that the overall absorption of N from the intestine was not improved by aureomycin, a finding which is in accordance with that of Pecora (*Jour. Nutr.*, **49,** 621, 1952) and a few others.

Since DEW ovomucoid inhibited growth even when fed with casein hydrolysate, it was considered worthwhile testing the action of aureomycin on this aspect. Experiments in which 0·01 g. per cent of aureomycin was fed with ovomucoid (one per cent) and casein hydrolysate (equivalent to ten per cent protein level), it failed to show any favourable effect on growth. Thus aureomycin counteracts only that part of the inhibitory action of ovomucoid which resides in its antitryptic activity.

Since the total digestibility of protein is not improved by aureomycin as mentioned above, it is difficult to envisage the mode of its action. Several hypotheses have been advanced by workers interested in the problem but none appears to be satisfactory. Further work alone can throw light on this interesting problem.

Physiological studies on purified ovomucoids.—The ovomucoids from DEW and HEW were isolated and purified as described by Narasinga Rao and Patwardhan (*Ind. Jour. Med. Res.*, **43,** 543, 1955) and their physio-chemical properties and chemical composition were studied in detail.

Both the ovomucoids are soluble in water and are not precipitated by the common protein precipitants, such as, heavy metal salts and acids, viz., trichloroacetic, glyoxalic, phosphomolybdic, and tannic acid. Flavianic and picric acids precipitated the DEW ovomucoid but not the HEW analogue. On addition of ammonium sulphate to the aqueous solution of ovomucoids, DEW ovomucoid was precipitated at 0·5 saturation, whereas the HEW ovomucoid required 0·78 saturation for complete precipitation. Thus there appeared some differences in the precipitation reactions. Both the ovomucoids were non-reducing, gave tests for the presence of carbohydrates and negative tests for SH groups. Other physico-chemical constants determined during the course of the investigation are given in Table III.

TABLE III.

Some physico-chemical constants of ovomucoids.

Particulars	DEW ovomucoid	HEW ovomucoid
Molecular weight (by osmotic pressure measurement)	38,500	37,000
Isoelectric point—pH	4·23	4·63
Optical rotation $[\alpha]^{21}_{D}$	–57°·88	–70°·94

Viscosity studies did not reveal any difference between the two proteins. Titration curves between pH 1·5 and 8·5 were also similar.

In a study of the chemical composition of the two ovomucoids, a qualitative detection of amino acids and sugars in ovomucoid hydrolysates was carried out by paper partition chromatography. Then the carbohydrates and some amino acids with special functional groups were determined either on intact or on hydrolysed protein.

The chromatograms of amino acids of DEW and HEW ovomucoids showed no qualitative differences. In both, the basic amino acid spots were weaker than the others and acidic amino acid spots were prominent. There were, however, certain marked quantitative differences as was confirmed by actual estimations. The DEW ovomucoid contained more of methionine and tyrosine than the HEW ovomucoid, whereas with regard to arginine it was the reverse. The HEW ovomucoid also contained much higher amounts of mannose and glucosamine than did the DEW ovomucoid. How far these differences in the chemical composition are responsible for the differences in the biological properties of the two ovomucoids is a question on which further work needs to be done.

3. Protein deficiency.

The effect of protein depletion and calorie restriction in albino rats.—Srinivasan and Patwardhan (*Ind. Jour. Med. Res.*, **43,** 1, 1955) have studied the effects of protein depletion on the activities of various enzymes in liver and plasma. They had observed that the reduction in enzyme activity was more severe in young growing animals than in adult rats. This study was of significance in view of the ætiological rôle of protein malnutrition in kwashiorkor. In this disease the state of protein deficiency is often complicated by the concurrent calorie deficiency although some observers are of the view that kwashiorkor results from a disproportion between total calories and protein calories. In view of this controversy it was considered worthwhile determining the influence of calorie restriction on the effects of protein depletion in albino rats.

A large number of young (five to six weeks old) rats were divided into four groups and given diets as follows: Group I: Diet A, containing 20 per cent casein fed *ad lib.* Group II: Diet B, containing 40 per cent casein fed in quantities equivalent to half of that consumed by the animals of Group I. Group III: Diet C, containing two per cent casein fed *ad lib.* Group IV: Diet D, containing four per cent casein but fed as to group II. Thus, the above treatment ensured comparable intakes of protein in groups I and II on the one hand and groups III and IV on the other while the calorie intake was restricted in groups II and IV to 50 per cent of that obtaining in animals of groups I and III. The rats were caged individually and weighed once a week. A certain number was sacrificed at 14 days intervals, the last batch being killed on 56th day from the start of the experiment. Liver, small intestine and pancreas were removed immediately after killing the animal. Liver and pancreas were weighed and homogenized. The small intestine was mostly free of food material since food was invariably withdrawn 20 hours prior to the killing of the animal. It was, however, split open longitudinally and gently rinsed with saline before homogenizing. The following estimations were done on the homogenates: (a) Liver—total N, succinic dehydrogenase, transaminase; (b) pancreas—total N, lipase; (c) small intestines—total N, succinic dehydrogenase, transaminase, alkaline phosphatase, esterase.

The rats on 20 per cent casein diets fed *ad lib.* grew very well. In group II there was retardation of growth, whereas rats in the low protein groups lost weight throughout the period of experiment. Calorie restriction at high protein intake did not have any effect on the various characteristics studied in liver, small intestine and pancreas. On the other hand, certain marked effects were seen in protein-depleted animals.

Liver.—In rats kept on two per cent protein diet fed *ad lib.* the liver weight was higher than normal. Total N in liver decreased markedly in the first 14 days but remained steady thereafter. The activity of succinic dehydrogenase and transaminase in liver showed a fall which was more marked in the case of the former enzyme. The superimposition of calorie restriction seemed to reduce the severity of these changes and delay their onset.

Intestine.—Total N, transaminase, alkaline phosphatase and esterase were not affected by protein depletion with or without calorie restriction. In protein-depleted rats succinic dehydrogenase activity was reduced; the effect was not more severe in calorie restricted animals.

Pancreas.—Pancreatic lipase activity was reduced in protein-depleted rats as was expected. Calorie restriction had no further effect.

The above results indicate that the added effects of calorie restriction superimposed on protein depletion are variable. Whereas in the small intestine and pancreas they were not noticeable at all, calorie restriction appeared to be beneficial so far as the liver was concerned. Further, it can also be said that within the limits of the present investigation small intestine, which is an organ with a high metabolic activity, appeared to be not so markedly affected by protein depletion as the liver and the pancreas. Further work is in progress.

4. PROTEIN METABOLISM.

Utilization of wheat protein in situ *and of separated wheat gluten.*—Phansalkar and Patwardhan (*Ind. Jour. Med. Res.*, **43,** 265, 1955) had suggested that the differences in the rates of utilization of animal and vegetable protein observed by them could at least partly be explained by the readiness with which protein contained in animal cell and vegetable cell membranes, respectively, was susceptible to attack by digestive enzymes. The vegetable cell membrane being more resistant allowed the protein contained in it to be attacked more slowly than was the case with animal protein. In order to test the validity of this concept, healthy subjects were given test meals prepared from (a) wheat flour, and (b) mixture of wheat gluten and wheat starch, respectively, and changes in blood amino nitrogen and urinary N were studied at intervals according to the technique already described (*Ann. Rep.*, 1953-54, p. 5). The results are summarized in Table IV.

The results show that the absorption of wheat gluten was quicker than that of wheat flour protein, the peak of amino N being reached about two hours earlier on wheat gluten than on wheat flour. The observations on urinary N excretion in the absorptive stage were more or less similar to those previously described on the animal and vegetable protein test meals. A quicker and larger excretion of urinary N was found in wheat gluten than in wheat flour test meal, the difference being most marked in the first four hours. Thus, these observations lend support to the hypothesis referred to at the beginning of this section.

TABLE IV.

Blood amino N and urinary N in wheat flour and wheat gluten test meals. (The values are averages for four subjects.)

Time in hours	WHEAT FLOUR TEST MEAL:		WHEAT GLUTEN TEST MEAL:	
	Blood amino N mg./100 c.c.	Urine N mg.	Blood amino N mg./100 c.c.	Urine N mg.
0	7·5	...	7·6	...
1	8·8	202	8·6	253
2	9·1	204	10·5	279
3	8·5	207	11·8	341
4	9·2	280	10·8	387
5	10·7	419	9·0	435
6	9·4	418	8·5	445
7	7·3	457	8·5	448

5. MAINTENANCE REQUIREMENTS OF PROTEINS IN YOUNG INDIAN ADULTS.

The question of minimum protein requirements, especially those of adults has been the subject of controversy since the days of Voit, who suggested the standard of 118 g. protein for a 70 kg. man. Considerable work has been done on the subject during the last fifty years without any agreement being reached. This is mainly due to the fact that there are no agreed criteria for determining the minimum protein requirements of adults. Usually, the minimum requirement is equated with the amount required to make, good the endogenous loss of nitrogen. Various investigators have used this criterion experimentally to determine the level at which adult subjects are in nitrogenous equilibrium and have found levels of protein varying from 24 to 65 g. per day depending upon the nature of the dietary proteins (Martin and Robison, *Biochem., Jour.*, **16,** 407, 1922; Hegsted *et al.*, *Jour. Lab. Clin. Med.*, **31,** 261, 1946; Bricker *et al.*, *Jour. Nutr.*, **37,** 163, 1949). Attempts were made to study the level at which N equilibrium could be attained in young Indian adults on diets containing animal and vegetable proteins, respectively.

Diets.—Experiments on nitrogen metabolism were conducted on five healthy young adult male volunteers varying in age from 21 to 27 years from among the scientific staff of the laboratories. The levels of protein, total calories, fat and carbohydrates of the diets used by us in these experiments were comparable to those used by Murlin *et al.* (*Jour. Nutr.*, **16,** 249, 1938). Each subject was started on diet I in which whole egg formed the source of dietary protein. After the completion of observations on urine and fæces, as described later, the subject was transferred to diet II or diet III in both of which vegetable protein replaced the egg protein. Total calories, protein and fat in all the three diets were almost the same.

The proportions of the total dietary proteins represented by egg protein and test protein were 75 to 80 per cent, the remainder being derived from other dietary constituents. In diet II the vegetable (test) protein was derived from a mixture of rice, pulse, and whole wheat flour, while in diet III it was derived only from refined wheat flour of 70 per cent extraction. The necessity of using these two different sources of vegetable protein in the experiments arose because of the fact that Patwardhan *et al.* (*Ind. Jour. Med. Res.*, **37,** 327, 1949) had used a mixture of cereals and a pulse as the main source of vegetable protein in their diets while Murlin *et al.* (*loc. cit.*) had replaced the egg protein by a single cereal protein under study. Our object in using the single cereal as the source of vegetable protein in the present series of experiments was further to compare the results obtained under conditions somewhat similar to those used by Murlin and his associates.

All the articles of diet were weighed every day, the amounts divided into three suitable portions, cooked and fed in the laboratory three times a day under supervision. When more than one subject was under the experiment at the same time the diet for each subject was cooked separately. Diets were analysed frequently for total nitrogen intake.

Urine and fæces.—Twentyfour-hour samples of urine were collected over 10 c.c. of ten per cent acetic acid and analysed for total nitrogen. These collections of urine continued till a steady state of urinary N excretion was reached. After this, 24-hour collections of both urine and fæces were made for four consecutive days and analysed for total nitrogen by Kjeldahl method. Creatinine was estimated in the urine by Folin's method which served as a check for the completeness of the sample.

The average figures for daily urinary and fæcal N in four-day balance periods on different diets in individual subjects are given in Tables V and VI.

It will be seen from Tables V and VI that when whole egg protein was replaced by a mixture of cereal and pulse proteins, the level of urinary N fell and that of fæcal N registered a rise. It was interesting to note, however, that the body was still in positive N balance. On the other hand, when wheat protein alone replaced whole egg protein, the urinary N rose, and the body went into negative balance; the fluctuation in fæcal N did not follow any particular pattern. With wheat protein, therefore, our observations are in line with those reported by Murlin *et al.* (*loc. cit.*).

From these Tables it will be clear that the average biological value (B.V.) and egg replacement value (E.R.V.) of vegetable protein derived from the composite mixture of cereals and pulse were 111 (range 99-118) and 103 (range 100-107), respectively, whereas those for the vegetable protein derived from refined wheat flour alone were 86 (range 84-88) and 88 (range 78-96), respectively. Our values for wheat flour agree well with the values reported by Murlin and Mattill (*Jour. Nutr.*, **16,** 15, 1938) and Sumner *et al.* (*Jour. Nutr.*, **16,** 37, 1938). It should be clear, therefore, that N metabolism in Indian adults studied by us was strictly comparable with that of Murlin's subjects who were habituated to high level of N metabolism on account of a high N intake. The subjects of Murlin and co-workers were in N equilibrium at an intake of 73 to 80 mg. N per kg. body weight and 3·2 to 3·8 g. per sq. m. body surface, whereas our subjects were in N equilibrium at 88 to 150 mg. per kg. body weight and 3·6 to 4·5 per sq. m. body surface. This apparent discrepancy can be ascribed

TABLE V.

Results of N balance experiments on whole egg (I) and mixed cereal (II) diet.

(Figures are averages of four 24-hour collections.)

SUBJECT	BSN (1)		SVP(1)		SGK(1)		JGJ		SVP(2)	
Diet period	I	II	I	II	I	II	I	II	I	II
Body weight, kg. ...	51·2	51·2	58·0	58·0	45·3	45·3	60·2	59·3	61·6	61·6
Dietary N, g. ...	6·82	6·86	6·82	6·86	6·27	6·51	6·27	6·20	6·27	6·20
Fæcal N, g. ...	1·54	1·86	2·28	2·95	1·95	1·92	1·60	2·40	1·71	2·60
Total urinary N, g. ...	5·19	4·54	4·28	3·55	3·83	3·89	4·96	4·10	4·46	3·47
Nitrogen balance, g. ...	+0·09	+0·46	+0·26	+0·36	+0·49	+0·70	—0·29	—0·30	+0·10	+0·13
Biological value, per cent	100	110	100	112	100	99	100	116	100	118
Egg replacement value, per cent	...	107	...	102	...	104	...	100	...	101

TABLE VI.

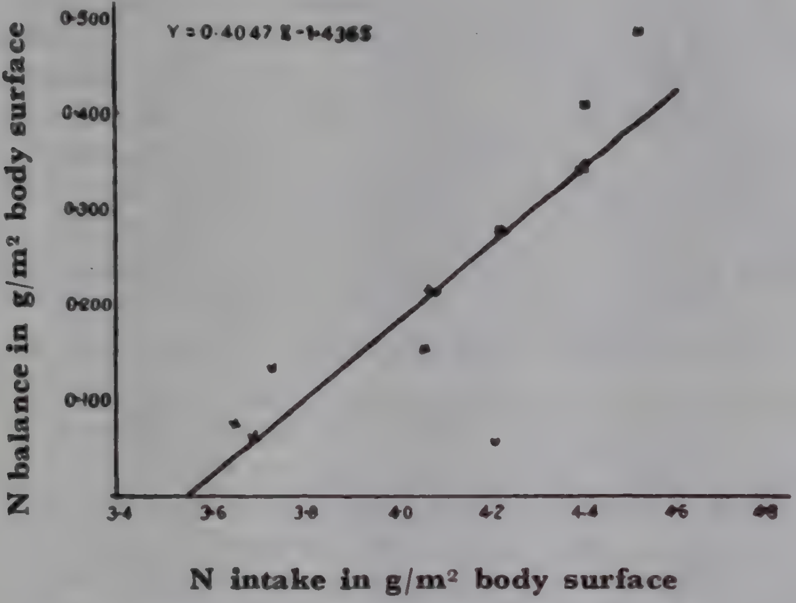
Results of N balance experiments on whole egg (I) and wheat flour (III) diets.

(Figures are averages of four 24-hour collections.)

Subjects	BSN(2)		SVP(3)		SGK(2)		HPT	
	I	III	I	III	I	III	I	III
Body weight, kg. ...	51·2	51·2	58·0	58·0	44·8	45·3	41·2	41·2
Dietary N, g. ...	6·27	6·48	6·27	6·52	6·27	6·52	6·27	6·52
Fæcal N, g. ...	1·62	1·87	2·17	2·00	1·93	1·31	1·92	1·83
Total urinary N, g. ...	4·78	5·80	3·87	4·65	3·73	4·81	3·22	4·12
Nitrogen balance, g. ...	—0·13	—1·19	+0·23	—0·13	+0·61	+0·40	+1·13	+0·57
Biological value, per cent	100	84	100	88	100	85	100	86
Egg replacement value, per cent	...	78	...	92	...	96	...	88

to a higher calorie intake by our subjects relatively to their body weight in comparison with their American counterparts. As a result, more protein per unit weight was consumed by the lighter Indian subjects.

The present series of observations has been utilized to determine the minimum protein requirements of our subjects. When the positive nitrogen balances observed at different nitrogen intakes both expressed as g. N per sq. m. of body surface were subjected to statistical analysis a high correlation coefficient ($\gamma = 0{\cdot}8406$) between the two was obtained. The results are plotted in the Figure.



The relation between nitrogen balance and nitrogen intake on egg protein and mixed cereal pulse diet.

The intercept on the x-axis shows the level of nitrogen intake per square metre body surface required to maintain nitrogen equilibrium. This figure is 3·55 g. per sq. m. which works out to 38·2 g. of protein (from cereal and pulse mixed) per day per adult weighing 70 kg. and 5 ft. 6 ins. in height. The value obtained by Hegsted *et al.* (*loc. cit.*) is 3·1 g. of N per sq. m. or 33·3 g. of protein per day per adult using all vegetable protein diet. The present observations on wheat flour diet are too meagre to permit their analysis in the same manner. They, however, indicate 46 g. as the approximate requirement of wheat flour protein. Further work on determining protein requirements based on single cereal and in mixture with pulses is in progress.

III. STUDIES ON FATS.

(*Under the auspices of the Council of Scientific and Industrial Research.*)

The presence of four or five octadecanedioic acids in cow and buffalo butter fat had been demonstrated by Phatak and Patwardhan (*Nature*, **172,** 456, 1953) using chromatographic separation on paper of the dibasic acid fraction obtained on acetic acid permanganate oxidation of the above fats according to the technique developed by Phatak, Mahadevan and Patwardhan (*Curr. Sci.*, **21,** 162, 1952). The same technique has been extended to the study of animal body fat, liver fats and milk fats of sheep and goat and human beings. The occurrence of at least four octadecanedioic acids in these natural fats could be demonstrated.

The next stage in the investigation was to know the relative proportions of these acids as they occurred in natural and hydrogenated fats. The paper chromatographic method was found unsuitable for quantitative separation and estimation of the dibasic acids. A method was, therefore, developed for quantitative partition chromatography with the use of silica gel. The aqueous and benzene phases of a well-shaken mixture of suitable proportions of water, ethanol, methanol and benzene were employed as the stationary and eluting phases, respectively. As the acids get eluted, the eluate is collected in fractions of suitable volumes and titrated against standard alkali. A plot of the volume of alkali consumed against the number of fractions shows the elution of the acids at well-defined intervals, the acids being eluted in the decreasing order of their C-chain lengths. The volume of the alkali needed to neutralize each of the bonds is also a quantitative measure of the acid comprising it. Further, the concentration of the neutralized eluate fractions yields a sodium salt from which the acids themselves can be liberated by acidification. The acids can further be indentified by treatment with reagents, such as p-bromophenacyl bromide.

The column used in the present experiments was standardized with a mixture of pure sebacic, azelaic and suberic acids. The order of elution of the acids was confirmed by the recovery and identification of the acids as mentioned above. After repeated runs had indicated that from 90 to 100 per cent recoveries could be consistently obtained the procedure was adopted for the separation of the dibasic acids obtained by the oxidation of ‘ iso-oleic ’ fraction of vanaspati. The results of two such runs are given in Table VII.

TABLE VII.

Composition of the mixture of dibasic acids in iso-oleic acids fraction of vanaspati.

	Exp. 3 Per cent	Exp. 4 Per cent
Dodecanedioic acid	5·7	5·3
Undecanedioic acid	7·4	6·9
Sebacic acid	18·6	18·9
Azelaic acid	56·8	57·7
Suberic acid	11·5	11·2
	100·0	100·0

Suberic, azelaic and sebacic acids have been isolated and identified as p-bromophenacyl derivatives. The quantities of undecane- and dodecane-dioic acids were small. Although the order of elution leaves little room for doubt about their identity, conclusive evidence would be desirable. Attempts to obtain these acids in larger quantities and their identification are in progress.

One can tentatively conclude from the quantitative separation studies described above that in the hydrogenated fat known as vanaspati, the $\triangle^{-8}$ and $\triangle^{-10}$ monoethenoid acids predominate next to the *trans* isomer of oleic acid, i.e., elaidic acid. If it is assumed that the undecanedioic acid present in the mixture of dibasic acids results solely due to the oxidation of vaccenic acid present in the fat, the content of the latter in the sample of vanaspati investigated in these studies can be estimated to be of the order of seven per cent of the total iso-oleic acid content.

Analyses of the iso-oleic acid fraction of a number of fats of both animal and vegetable origin employing the present method are in progress.

IV. RICE DIET AND FERTILITY.

Ranganathan (*Curr. Sci.*, **23,** 3, 1954) has pointed out that an examination of census figures for population increase or of birth rates for different provinces of India or for different countries in the world failed to show any clearcut association between rice diets and fertility. Mathen (unpublished) arrived at nearly the same conclusion after examining the pattern of fertility rates in the various countries of the world and the different regions within India. While the data on human population may indicate the trend, they cannot afford incontrovertible evidence on the effect of diet *per se* on fertility rates for several factors other than diet complicate the issue. Animal experiments under rigidly controlled conditions are essential to determine the rôle of diet only, uncomplicated by other factors. Observations on albino rats made during the course of the last year are given below.

The experiments were conducted under two broad heads: (*a*) short term studies to determine whether ' rice diets ' *per se* have any influence on birth rate, litter size, etc., and (*b*) long term studies to ascertain the rôle of ' rice diets ' in influencing the fertility rate over generations. The diet used in these experiments was stock diet which has been found adequate by experience at Coonoor for the maintenance of a healthy rat colony. The cereal component was varied for the purpose of the present experiment. Ten pairs of stock rats, 120 days old, one pair in each cage, were used for mating. Five pairs were maintained on ' rice diets ' and the other five on wheat diets. The cereals formed 60 per cent of the dry weight of the diet. Both the (rice and wheat) diets furnished 416 calories and 6 g. fat per 100 g. The rice diet provided 10·6 per cent protein and the wheat diet 15·1 per cent protein and more than adequate amounts of minerals and vitamins were provided by these diets.

When litters were cast, the young ones above six numbers in each litter were sacrificed and the rest weaned after 21 days. The mothers were allowed rest for one month in between litters when they were again mated with the same male for the second, third and the fourth matings. Records were maintained of the weekly weights of the parent rats, litter size, birth-weight of young ones, sex ratio, inter-breeding period, weight at weaning, growth rate of the young ones, etc. The short term studies have yielded the following results:

1. No difference was found in the fertility rate of animals maintained on ' rice diets ' and ' wheat diets '.

2. There was no difference in the growth rate of the animals in the two groups, in spite of the ' rice diet ' furnishing a smaller quantity of proteins, about 30 per cent less than that furnished by the ' wheat diet '.

3. There was also no significant difference in the litter size in the two groups.

4. The birth-weight on the 'wheat diet' was greater by about 25 per cent than that on the 'rice diet' but this initial advantage disappeared within a few weeks of weaning.

5. There was no difference in the inter-breeding period of the animals in the two groups.

Long term studies were carried out with the young born at the first mating in the two groups when they were 120 days old. The experimental technique was the same as that adopted in the short term studies. The experiments are still under way.

V. NUTRITION CLINIC.

During the year 4,362 cases attended the Clinic. Of these, 224 cases were found to be suffering from nutritional disorders. The distribution of nutritional diseases is given in Table VIII :—

TABLE VIII.

Statement of cases seen during the year ending 31*st March*, 1955.

Type of deficiency	Presenting features	Inpatient	Outpatient
Vitamin A	Night blindness only ...	...	13
	Xerosis and/or Bitot's Spots ...	...	44
	Keratomalacia	2	3
Mixed deficiency	B-complex deficiency		
	(*a*) Burning feet syndrome ...	...	9
	(*b*). Angular stomatitis, cheilosis and glossitis	...	41
	(*c*) Pellagra	...	2
	Nutritional œdema ...	8	...
	Nutritional œdema syndrome ...	44	17
	Undernutrition	...	39
Rickets ...		...	2
	TOTALS ...	54	170

Total attendance for the year ... 4,362

VI. CLINICAL INVESTIGATIONS.

A. STUDIES ON NUTRITIONAL ŒDEMA SYNDROME (KWASHIORKOR).

1. Steatorrhœa in kwashiorkor.

A large number of cases of kwashiorkor investigated at Coonoor showed steatorrhœa; the fæces showed plenty of fat globules, fatty acid crystals and soap. The occurrence of steatorrhœa in kwashiorkor has been reported from other parts of the world also. However, a perusal of the voluminous literature on the disease reveals very few studies of steatorrhœa. In the present work an attempt has been made to investigate this problem.

Six cases of kwashiorkor with ages ranging from 18 to 33 months were taken up for this study. The clinical features of these cases conformed to the description already given (*Ind. Jour. Med. Res.*, **42,** 555, 1954). All the cases showed abundant fat globules in the fæces under the microscope, on the day of admission. The subjects were put on the different experimental diets the main variable in which was the fat content. Each experimental diet was administered for periods ranging from three to nine days. The total duration of observation of the subjects ranged from 21 to 28 days. Unlike in cœliac disease where longer periods of observation on different diets are possible because of the chronic nature of the disease, in kwashiorkor the observation periods are necessarily restricted by its dynamic nature. Twentyfour-hour fæcal collections were made daily for the entire duration of the experiment. Estimations of total fat, fatty acids and the iodine number were carried out for all the fæcal samples. Similar determinations were made on the experimental diet as well. Five cases were followed up and fat balance studies were repeated on them a month after their discharge from the hospital. Some observations emerging from the study are summarized below:

(*a*) On a diet based on skim milk and supplying minimal amounts of fat, the subjects were found to excrete in the fæces, amounts of fat considerably larger than the intake. This would indicate that a proportion of fat in the fæces in kwashiorkor is of ‘ endogenous ’ origin. Considering the high percentage of neutral fat in the fæces on this diet, it would appear that the ‘ endogenous ’ fat was made up of fatty acids and neutral fat.

(*b*) When glucose was added to the above diet, fæcal fat showed an increase in all the subjects investigated. This would point to an aggravating effect of glucose on steatorrhœa—an observation of theoretical and practical interest. The mechanism underlying this effect needs further investigation.

(*c*) On two high fat diets which contained nearly equal amounts of fat but in one of which the fat was derived from vegetable sources and in the other (diet 6) from butter, it was observed that in 5 out of the 6 cases investigated the steatorrhœa was more marked on the vegetable fat diet than on the butter fat diet. In only one case, no difference in response to the two diets was noticed.

(*d*) Unlike in cœliac disease, wheat diet did not have an appreciable aggravating effect on the steatorrhœa in two cases of kwashiorkor which were investigated for this purpose.

(*e*) In two cases in which 10 mg. folic acid a day were administered for four days no appreciable beneficial effect was observed.

(*f*) The severity of the steatorrhœa bore no relationship to the clinical severity of the case. In five cases which were reinvestigated a month after discharge, it was observed that in spite of considerable clinical improvement in all cases, improvement with regard to steatorrhœa could be demonstrated only in two cases. The persistence of steatorrhœa after most clinical and biochemical lesions had been reversed is of considerable interest.

2. *Treatment of kwashiorkor with vegetable protein diet.*

(*a*) *Use of body composition studies on animals to evaluate vegetable protein diets in the treatment of kwashiorkor.*—In the previous report, the results of treatment of a series of cases of kwashiorkor with a vegetable protein (Bengal gram) diet were presented. It appeared from the clinical response that the Bengal gram diet was as effective as the milk diet but the regeneration of plasma albumin was found to be much slower with the Bengal gram diet as compared to that obtained with the skim milk diet. The possibility that the clinical criteria used to assess the relative efficacy of the different therapeutic regimens were unable to discern differences regarding tissue regeneration on the two diets had to be considered. The extremely dynamic nature of the disease, the fact that both the lines of treatment constituted vast improvements over the preceding home diets of these patients and the relatively short duration of observation are factors which might be expected to vitiate the validity of purely clinical assessment of differences in therapeutic response. It was considered worthwhile, therefore, to determine the relative efficacy of these two dietary regimens for the building up of various body components in protein-depleted experimental animals. The results of such an investigation would incidentally provide information on the basis of which the Bengal gram diet could be improved for the treatment of protein depletion.

Thirty-six rats weighing between 49 and 65 g. were fed for 15 days a low protein low calorie diet similar to the type of diet consumed by the children developing kwashiorkor. At the end of this period, 20 rats were killed and their body composition determined. Sixteen remaining animals were divided into four groups of four animals each and placed individually in metabolic cages. Each group received one of the following four diets for a period of 21 days.

Diets.

	1		2	3	4
Skim milk powder, g.	30	Bengal gram, g. ...	61	48	48
Dry bread powder, g.	40	Rice, g. ...	...	52	52
Starch, g. ...	30	Dry banana, g. ...	6	...	...
		Jaggery, g. ...	5	...	...
		Starch, g. ...	28	...	...
		Calcium lactate, g. ...	...	...	1

All the diets contained 14 per cent of protein. Diet 1 was moistened and steamed, whereas diets 2 to 4 were moistened and autoclaved at 15 lb. for 15 minutes before being offered to rats.

Quantitative collections of urine and fæces were made with usual precautions. A week's collections were pooled and analysed. At the end of the experimental period the animals were sacrificed for the determination of their body composition.

The animals were sacrificed and their intestinal contents were removed, the carcass was cut into numerous small pieces, dried in an air oven to a constant weight. The dried carcass was ground to a fine powder. Water was estimated as the difference in the carcass weight before and after drying, fat was determined after ether extraction, nitrogen by the Kjeldahl method, minerals by ashing and cell solids as the difference between the dry weight of carcass on the one hand and the weight of fat and minerals on the other.

The results are presented in Table IX. It would be seen that the Bengal gram was in fact inferior to skim milk in the matter of regeneration of ' cell solids ', fat and minerals. When supplemented with calcium lactate the gain in minerals on Bengal gram was comparable with that obtained on skim milk diet. Studies on essential amino acid composition of Bengal gram andrice carried out in these laboratories (*Jour. Nutr.*, **51,** 261, 1953) had indicated that a combination of these two would be beneficial. When Bengal gram and rice were combined in the proportion used in the experiment (diet 3) it was noticed that the increase in cell solids for every g. of N retained was greater on the mixed diet than with Bengal gram alone and nearly as great as that with skim milk. However, when the increment in cell solids was calculated on the basis of N intake then the differences between diets 2, 3 and 4 were negligible; this was because the addition of rice in the proportion used in the experiment actually lowered the nitrogen retention and was, therefore, not associated with any overall beneficial effect. Comparative clinical trials with Bengal gram and Bengal gram and rice carried out on kwashiorkor cases would appear to confirm this observation. It is possible that combinations of rice and Bengal gram in other proportions would be more beneficial.

The studies have incidentally also revealed remarkable differences in the build-up of fat on the different diets. For the deposition of 1 g. fat, animals on diets 1, 3 and 4 needed significantly less calories than those on diet 2. It would appear that the quality of dietary protein could influence the deposition of fat on isocaloric diets.

(*b*) *Clinical trials with vegetable protein diets in kwashiorkor.*—In the last annual report results of treatment of 34 cases of kwashiorkor with skim milk and of 35 cases with vegetable protein diets were compared. A large number of patients were further investigated on the two diets and a third group was treated with a diet containing Bengal gram, rice and calcium lactate. This diet was nearly identical to the Bengal gram diet with regard to protein and calorie content. The results of clinical appraisal showed that the Bengal gram-rice-calcium lactate diet was not superior to the Bengal gram diet, a conclusion borne out by the results of animal experiments presented above. It is probable that better results might be achieved by using combinations containing different proportions of these ingredients.

3. *Role of maternal malnutrition in kwashiorkor.*

It is generally agreed by most workers engaged in investigations on kwashiorkor that the incidence of the disease in early infancy is negligible. A series of 207 cases investigated here (*Ind. Jour. Med. Res.*, **42,** 555, 1954) did not include a single infant less than ten months age. The absence of cases in early infancy has been interpreted as indicating that in the development of the disease, malnutrition during the weaning and post-weaning stages plays the dominant part. At the same time, a probable rôle of maternal and consequent neonatal malnutrition predisposing to the occurrence of the disease has been often suggested. Studies of maternal and neonatal conditions in relation to the incidence of kwashiorkor are

TABLE IX.

Body composition on the experimental diets after 21 days of rehabilitation.

(The values are averages of four animals in each of the four groups.)

Diet	N intake g.	N absorbed g.	N retained g.	GAINS DURING THREE WEEKS, IN G. IN:										Cals/g. increase in fat
				Body weight		Body water		Cell solids		Minerals		Fat		
				Total	Per g. N retained	Total	Per g. N retained	Total	Per g. N retained	Total	Per g. N retained	Total	Per g. N retained	
Group I skim milk	6·63	5·87	4·38	77	17·8	52·8	12·1	12·9	2·9	2·2	0·5	9·1	2·0	130
Group II Bengal gram ...	6·09	5·15	4·07	49	11·9	35·7	8·8	7·4	1·8	0·6	0·15	5·3	1·2	236
Group III Bengal gram and rice ...	5·06	4·31	2·23	33	14·5	22·1	9·8	5·9	2·6	0·5	0·23	4·5	1·9	208
Group IV Bengal gram + rice + calcium lactate ...	5·22	4·41	2·61	39	15·1	24·2	9·4	6·4	2·5	1·3	0·49	7·1	2·6	145

obviously necessary to throw further light on this problem. The results of such studies carried out here are presented below.

The investigation included the following:

(a) Determination of birth-weights of (i) a series of 500 full-term infants (250 male and 250 female) born to women belonging to the poorest section of the population among which cases of kwashiorkor have been found to occur, and (ii) a series of 200 full-term infants born to women belonging to the well-to-do sections of the population.

(b) A careful trace-back of the large series of 207 cases of kwashiorkor investigated here with a view to obtaining birth-weights of such of them as were delivered in the hospital.

(c) A diet and nutrition survey of a group of 241 pregnant women, of different parities and in different trimesters of pregnancy belonging to the poorest section of the community, among whom cases of kwashiorkor are frequent. The survey also included the hæmoglobin and plasma protein estimations.

(d) A study of the 24-hour milk yield (through the test-feed technique) of 88 mothers in different stages of lactation, belonging to the poorest socio-economic groups.

The results are set out in Table X:—

Table X.

Clinical and biochemical findings in 200 antenatal cases.

Average calorie intake	1,211
Average protein intake, g.	35
Cases showing clinical signs in per cent	
Œdema	9·5
Vitamin A deficiency	14·5
B-complex deficiency	44·0
Burning feet	12·0
Calf tenderness	18·5
Per cent cases with serum proteins	
Over 7 g. per 100 c.c. serum	48
6 to 7 „ „ „	21
5 to 6 „ „ „	24
4 to 5 „ „ „	6·2
Less than 4 „ „ „	0·6
Per cent cases with hæmoglobin	
Over 12 g. per 100 c.c. blood	15
10 to 12 „ „	29
Less than 10 „ „	56

The salient conclusions may be listed as follows:—

(*i*) The mean birth-weight of infants of mothers belonging to the poorest socio-economic groups was 6·01 lb. and that of infants of mothers belonging to the well-to-do groups was 6·85 lb.

(*ii*) The mean birth-weight of infants who later developed kwashiorkor was 6·0 lb.; it did not significantly differ from that of other infants in the same socio-economic group.

(*iii*) The pregnant women investigated revealed a high incidence of malnutrition including protein malnutrition.

(*iv*) The average lactation performance of poor women varied between 21 oz. for 0 to 6 month period and 12 oz. for 18 to 24 month period. Considering her inadequate diet, the performance should be considered satisfactory.

(*v*) These studies do not indicate that maternal malnutrition is directly concerned in the etiology of kwashiorkor.

4. *Further observations on the pathogenesis of œdema in protein malnutrition.*

It has been maintained by Trowell and others that a high calorie/protein ratio apart from protein deficiency is important in the development of kwashiorkor. In the present investigation, the response of two groups of animals—one on high calorie low protein diet and the other on low calorie low protein diet—to water load test has been investigated.

Forty-four male rats (weighing at the beginning of the experiment 40 g. to 50 g.) divided into two comparable groups of 22 each, and 14 male monkeys divided into two comparable groups of seven each, were employed in the investigation. The low protein low calorie diet for the rats consisted of 4 g. of a diet containing one per cent of protein. The high calorie low protein diet was exactly similar to the low calorie low protein diet in all respects except that it supplied in addition tapioca starch *ad lib*., fed in the evening after the former diet was consumed. The low calorie low protein diet for the monkeys consisted of 75 g. of a diet containing 3·6 per cent of protein; the high calorie diet was identical to the low calorie low protein diet except that it supplied in addition tapioca starch *ad lib.* The dietary regimens were continued for periods ranging from two to three weeks in rats and four to six weeks in monkeys. The results indicated in Table XI would show that the animals on high calorie low protein diet showed a more abnormal response to the water load test than those on the low calorie low protein diet. Earlier reports from these laboratories that animals on a high calorie low protein diet showed a greater degree of fatty infiltration of their livers than those on a low calorie low protein diet would appear significant in this connection.

TABLE XI.

Response to water load tests of animals on low protein diets with low (l.c.) and high (h.c.) calorie intakes.

(Figures in Table are in per cent of administered water excreted.)

Animals	Diet	Number of animals	TIME IN MINUTES:						
			60	90	120	150	180	210	240
Rats	(a) l.c.	22	9·3	29	49	60	...	64	70
	(b) h.c.	22	3·7	15	28	38	...	56	64
Monkeys	(a) l.c.	7	9	16	36	52	60	66	70
	(b) h.c.	7	8	15	31	39	47	49	54

URINARY EXCRETION OF 17-KETOSTEROIDS IN NORMAL AND UNDER-NOURISHED SUBJECTS.

There are several reports on the urinary 17-ketosteroid excretion of normal subjects from Europe and America. However, observations on 17-ketosteroid excretion of Indian subjects are extremely scanty. Friedman (*Lancet*, **ii,** 262, 1954) reported values for 24-hour urinary 17-ketosteroid excretion in healthy Indian adults; figures for healthy Indian children are, however, not available. Observations on healthy adults and children are obviously necessary for a correct interpretation of the values for ketosteroid excretion observed in diseased states.

Keys and co-workers (*Biology of Human Starvation*, I, 755, 1950) have studied the excretion of 17-ketosteroids in the urines of semi-starved subjects. In general, however, the effect of malnutrition on 17-ketosteroid excretion has not received much attention. In the cases of nutritional œdema in adults investigated here (*Ind. Jour. Med. Sci.*, **6,** 277, 1952) evidence of adrenal and testicular damage was reported. These cases were also found to show 'low-normal' levels of sodium, sub-normal levels of chloride and 'high-normal' levels of potassium in the serum and the similarity with regard to some aspects of the clinical and electrolyte picture between nutritional œdema and Addison's disease was pointed out (*Metabolism*, **2,** 521, 1953). It was felt that a study of the urinary 17-ketosteroid excretion in cases of nutritional œdema syndrome (kwashiorkor) in children could throw further light on this question. Since data for normal Indian adults and children were lacking, it was found necessary also to investigate the 17-ketosteroid excretion in normal adults and children.

For the collection of normal values, nine male members of the staff of the laboratories with ages ranging from 20 to 45 years and twelve normal children (seven males and five females) with ages ranging from three to eight years were chosen. For the study of 17-ketosteroid excretion in undernutrition, six adult male cases of nutritional œdema and seven cases of nutritional œdema syndrome (kwashiorkor) with ages ranging from three to seven years were selected. This is obviously an unusually high age group for the general run of kwashiorkor cases; but cases in such high age groups were deliberately chosen to facilitate 24-hour urine collection. All cases of nutritional œdema in adults and nutritional œdema syndrome in children conformed to the clinical descriptions of these conditions previously reported. All cases were admitted into the hospital and complete 24-hour urine collections were made over 10 c.c. of concentrated HCl under strict supervision daily for four days prior to instituting any treatment. Similar collections were again made for four days at the end of a few weeks of a high protein diet treatment. In a few cases, collections of urine for four-day periods were also made more than once during the course of the treatment. The samples were kept in the refrigerator at 5°C. until taken up for analysis. The method employed for the estimation of 17-ketosteroids was that described by Warren and King (Methods in Medical Research, **II,** 314, 1950).

The results are set out in Table XII. The values for 17-ketosteroid excretion observed in the healthy male adults investigated here were in general lower than those reported for the English and American adults but were nearly the same as those reported by Friedman (*loc. cit.*) for Indian adults in Madras, by Lugg *et al.* (*Nature*, **174,** 1147, 1954) for Indian adults in

Malaya. The values obtained for healthy Indian children in the present investigation were also generally lower than the reported figures for children in Europe and America.

TABLE XII.

Urinary 17-ketosteroids excretion in health and undernutrition.
Healthy subjects.

Subjects		Age—Average and range years	Ketosteroid excretion, mg.	Range, mg.
Children	Males (6)	4·4 (3·5—6)	0·25	0·06—0·43
	Females(5)	5·8 (3—9)	0·60	0·21—1·80
Adults ...	Males (9)	32·4 (23—45)	7·94	3·0—13·0

Cases of undernutrition.

Disease	Age	Sex	Initial, mg.	Final, mg.	Interval between two estimations, days
Nutritional œdema	57	M	0·40	1·30	75
	36	M	0·80	4·10	88
	35	M	0·89	1·25	59
	55	M	0·20	...	Patient died on third day
	47	M	0·30	3·20	89
	38	M	3·69	4·61	24
Nutritional œdema syndrome	12	F	0·78	1·60	31
	4½	F	0·29	0·48	55
	6	F	0·09	0·15	54
	4½	F	0·10	0·11	25
	7½	M	0·20	0·49	32

The values of 24-hour urinary 17-ketosteroids excretion in adult cases of nutritional œdema at the time of admission were found to be far below those for healthy Indian adults. The values tended to rise during nutritional rehabilitation but even after 45 to 90 days of treatment the excretion was still considerably below the normal values. In children with nutritional œdema syndrome also, the 17-ketosteroids excretion on admission was lower than for healthy children of corresponding ages; but unlike in adults it was found to return to normal after a few weeks of nutritional rehabilitation. It would appear that whatever defect responsible for the lowered 17-ketosteroid excretion in undernutrition, it is more easily reversible in children than in the adult cases.

There was no correlation between the urinary 17-ketosteroids values and the clinical condition of the patient. Keys *et al.* (*loc. cit.*) have suggested a correlation between the urinary 17-ketosteroids and creatinine excretion. In the present series of cases no such correlation could be demonstrated.

VII. FIELD INVESTIGATIONS.

1. FURTHER STUDIES ON SKELETAL GROWTH IN INDIAN CHILDREN.

In the previous report, results of the examination of radiograms of 284 children were given. The present report deals with observations based on analysis of radiograms of 577 children. The results of a comparison between the chronological and the skeletal age of these children (as judged by American standards) are given in Table XIII:—

TABLE XIII.

Comparison between the chronological and skeletal age of 577 *Indian children in the Nilgiris.*

Economic status	Total examined	Chr. and skel. ages agree, as judged by American standards	Skeletal age deviating from chronological age by years, as judged by American standards					
			1	2	3	4	5	6
Well-to-do								
Number ...	201	157	21	20	3	...	...	...
Per cent ...	100	78·10	10·45	9·95	1·50	...	...	...
Rest								
Number ...	376	180	55	56	31	28	21	5
Per cent ...	100	47·87	14·62	14·89	8·24	7·45	5·58	1·32

It will be observed that among the children of the well-to-do class, the ossification centres of bones of the wrist and hand appeared in 78 per cent cases at about the same age as in American children, whereas in children from the lower economic strata their appearance was delayed by one to six years in 52 per cent of the children examined. In the present study 48 per cent of children of the poor group conformed to the American standards, while in the previous report only 26 per cent of children of the poor group did so. The reason for this may well lie in the fact that the present group included a very much higher percentage of younger children in whom the deviations from normal cannot be great. A much larger number of children belonging to each age group will have to be studied to gain a correct appreciation of the situation.

Skeletal development in kwashiorkor.—Skeletal examination of the poorer class also included 34 children suffering from kwashiorkor between the ages of nine months to six and a half years.

Definite retardation in the skeletal development was observed in 23 of these children. As judged by American standards, the skeletal deviation varied from five months to three and a half years. It may be pointed out that the extent of retardation in skeletal growth observed in kwashiorkor cases was not of a greater order than that observed in other children of the same socio-economic group. It was also interesting to observe that 11 out of 34 kwashiorkor cases did not show any evidence of skeletal retardation.

Transverse lines of growth arrest were observed in 70 children, 10 of whom belonged to the well-to-do classes. Every case of kwashiorkor treated at the Nutrition Clinic and later followed up showed the transverse lines of growth arrest. In a few other cases definite history of illness months or years earlier to taking the *x*-rays was obtained.

2. Regional variation and adequacy of Indian dietaries.

The reasons for major differences in the food habits of the poor people from different parts of the country must be sought mainly in the influence of climate, soil and natural resources on the availability of essential foodstuffs. The climate and soil conditions in different parts of India are so very different that they limit to a great extent the cultivators' selection of crops, and hence a variety of cereals, pulses and vegetables are produced and consumed by the people in different regions. Protective foodstuffs, such as milk and milk products, are found in negligible amounts in poor class family diets, and as cereals and pulses form the most economic source of food, the bulk of the calorie requirement is derived from these foodstuffs. The differences in the dietaries are traceable to the differences in the types of cereals and pulses consumed by the people. Due to differences in the composition of these foodstuffs, there may be differences in the consumption of nutrients as well. Each region will have its own nutrient intake pattern depending upon the consumption of different foodstuffs. It is natural to expect the prevalence of similar patterns in the neighbouring regions and places having similar conditions. In view of these considerations it was decided to analyse the published findings on diet surveys in order to compare the dietary patterns in different States with a view to evaluating their relative adequacy as judged by the intake of all the essential nutrients. It was also felt that such examination would throw light on changes in dietaries within a State if any could be found in repeat diet surveys.

Use was made of the information on 843 diet surveys published in the Special Report Nos. 20 and 25 of the Indian Council of Medical Research (1951, 1953). Additional information on 177 surveys conducted in different states between 1949-1953 was available in the annual publication of the Indian Council of Medical Research entitled ' Report of work done in nutrition in the States '. Thus, a total of 1,020 diet surveys from different parts of the country, and for the same State at two different periods was subjected to analysis. The 'distance' between the two States was considered to be the most suitable index for these comparisons. The principal components of the common correlation matrix were measured from a set of quantities considered adequate for the maintenance of good health (Recommendation of the Nutrition Advisory Committee on Dietary Allowances, 1944) in order to know whether the intake values were inadequate or not, and to compare sets of figures for the same State at two periods.

It was interesting to find that eleven States out of a total of sixteen arranged themselves into three groups. This grouping could be explained mainly on the basis of consumption of cereals. The first group composed of States where people consumed more wheat while in the second rice was found to be the most popular cereal. The third group might arise from the fact that both rice and wheat were consumed by the people. Moreover, millets, such as *cholam*, *cumbu*, etc., were also popular in the States of this group. The negative values of the scores obtained from the principal components of the common correlation matrix clearly indicated that the intakes of nutrients were at marginal levels in a few States and grossly inadequate in many of them. Delhi, Kashmir, Mysore and West Bengal came in the former category, while the remaining fell in the latter. In the second period under investigation there has been some improvement in all the States except Mysore.

Similarly, it was observed from the common correlation matrix that many of the nutrients were very closely related with each other. Except the correlation coefficients fat-carbohydrate (—0·0614)and calcium-riboflavin (0·0156) the rest were statistically significant. Some of the significant values of the correlation coefficients were quite evident, for example correlations of calorie value with protein (0·8002) carbohydrate (0·8241) and fat (0·4350). These very close correlations might arise from the fact that all foodstuffs usually consumed by the people contain many of the nutrients, and possibly due to natural aptitude on the part of man to select foodstuffs to make up deficiencies and to help metabolic processes. In support of the first contention the correlations vitamin A-vitamin C (0·8085), calcium-phosphorus (0·4234) and protein-carbohydrate (0·8052), could be mentioned, while for the second it was interesting to note the significant correlations fat-thiamine (–0·1529) and carbohydrate-thiamine (0·6360). The negative sign of the coefficient for fat-thiamine suggested that smaller quantities of thiamine intake were associated with larger quantities of fat to act as sparing agent for thiamine deficiency. Similarly, the positive sign of carbohydrate-thiamine coefficient showed that there was proportionate intake of thiamine to help metabolism of carbohydrates. It was thought that the negative correlation coefficients fat-nicotinic acid (–0·2059) and nicotinic acid-calcium (–0·1182) might suggest points for further laboratory investigations.

VIII. PROTEIN MALNUTRITION PROJECT.

(*Assisted by the World Health Organization*).

In December 1954, the World Health Organization placed some funds at the disposal of the Indian Council of Medical Research for work to be conducted at the Nutrition Research Laboratories on the subject of protein malnutrition. The investigation was to include:

(i) A survey of the extent and severity of protein malnutrition in children,

(ii) collection of information on foods utilized in diets of young children,

(iii) studies on the use of low cost protein rich foods of vegetable and animal origin, mainly the former, for treatment and prevention of protein malnutrition, and

(iv) methods of preparation of foods as supplements to children's diets, etc.

The investigation envisages work in the laboratory, clinic and the field with a view to finding a satisfactory solution of the problem of protein malnutrition. Work on items (i), (ii) and (iii) has already commenced along the lines described below and has been in progress for some time now.

Evaluation of nutritive value of vegetable proteins for growth and tissue building purposes.—Cereals, pulses and leafy vegetables are being investigated in various combinations. Attempts are being made to determine in young albino rats, the growth efficiency ratio of mixed proteins and their capacity to regenerate hæmoglobin and plasma proteins. The results obtained thus far indicate that mixing of pulses and leafy vegetables with cereals in certain proportions enhances the growth-promoting capacity of the protein mixture considerably. Further observations on this aspect as also on plasma protein and hæmoglobin regeneration are in progress.

Treatment of kwashiorkor with vegetable protein diets.—Results of treatment of kwashiorkor with Bengal gram and Bengal gram-rice-calcium lactate diets are described on page 42 of this report.

Survey of protein malnutrition.—A diet and nutrition survey in one urban and one rural community in Madras State has recently been completed. Information on (*a*) heights and weights of infants and children up to five years of age, (*b*) supplementary feeding of infants and weaning practices, (*c*) blood hæmoglobin levels, (*d*) antenatal nutritional status of women, and (*e*) lactation performance of mothers from 0 to 24 months after parturition, etc., has been collected and the data are being examined. Surveys in other regions of South India are contemplated.

2. Nutrition Research Unit under Dr. B. C. Guha at the University College of Science & Technology, Calcutta.

I. STUDIES ON THE BIOSYNTHESIS OF ASCORBIC ACID.

Synopsis of the work.—The enhanced urinary excretion of ascorbic acid by rats under chloretone can be completely suppressed if the animals are made either folic or pantothenic-acid deficient. The action of narcotic can be restored by administering these vitamins again. The ascorbic-acid reserves in different tissues of the rat are appreciably reduced under folic or pantothenic-acid deficiency. A daily supplement of ascorbic acid to the rats deficient in either of the above vitamins can significantly prolong the survival period of the animals.

Effects of the deficiency of some B-vitamins on the reserves of phosphate-esters in the liver tissue of rats have been studied. It has been found that inorganic phosphorus increases and organic phosphorus decreases when the rats are rendered deficient in thiamin, riboflavin or folic acid.

The *in vitro* experiments with tracer compounds, such as sodium pyruvate-2-C-14 and sodium acetate-1-C-14, are now in progress.

The work has been directed mainly along the following lines:

(A) Factors influencing the biosynthesis of ascorbic acid.

(B) Regarding the precursor of ascorbic acid.

A. FACTORS INFLUENCING THE BIOSYNTHESIS OF ASCORBIC ACID.

(*i*) *Effects of deficiency of folic and pantothenic acids on the biosynthesis of ascorbic acid by the rat.*—It has been reported before that the biosynthesis of ascorbic acid by the rat is dependent on some vitamins of the B-group, particularly thiamin and riboflavin (Roy, Roy, and Guha, *Nature*, 1946, **158,** p. 238). There are reports (Nichol and Welch, *Proc. Soc. Exper. Biol. Med.*, 1950, **74.** p. 52; *Ibid*, 1950, **74,** p. 398) that ascorbic acid takes some part in the conversion of folic acid to the *Leuconostoc citrovorum factor* (LCF) and that ascorbic-acid level in liver tissue of rats is greatly diminished by the administration of aminopterin, an antifolic acid compound. It thus appears that there is a relation between folic acid and ascorbic acid metabolism in the rat.

Again, co-enzyme A, one component of which is pantothenic acid, has been found to be closely involved in pyruvic acid oxidation. It has also been reported before that chloretone brings about an inhibition of pyruvic acid oxidation (Roy, Roy and Guha, *Ann. Biochem. Exper. Med.*, 1951, **11,** p. 73) and at the same time stimulates ascorbic-acid synthesis in the rat. So, it was of some interest to study the deficiency of each of these vitamins on the urinary excretion of ascorbic acid by the rat under chloretone.

(*a*) *The effect of folic or pantothenic-acid deficiency on the urinary excretion of ascorbic acid by rats under chloretone.*

Experimental.—In these studies, male albino rats, weighing 150 g. to 200 g. and 40 g. to 60 g., were used, respectively, for folic and pantothenic-acid deficiency experiments. The group on folic-acid deficiency experiments was given aminopterin in the basal stock diet (4 mg./kg.). The group on pantothenic-acid deficiency experiments was given a synthetic diet (Nelson and Evans, *Proc. Soc. Exper. Biol. Med.*, 1947, **66,** p. 299), containing all the B-vitamins except pantothenic acid. The rats were placed in metabolism cages and fed the basal diet for 4 days in order to get them used to the synthetic diet and to the metabolism cages. After this period all the rats were divided into groups of 12. Each group on the deficient diet had corresponding pair-fed control group which was supplied with the vitamin in question. When signs of deficiency appeared in the rats, all the groups, both deficient and control, were fed with chloretone daily (15 mg./100 g. body-weight). The groups on the deficient diets were later given the appropriate vitamin in order to study its effect on vitamin C excretion.

The urine excreted by the rats was allowed to drain into a solution of ten per cent oxalic acid and a daily estimation of urinary ascrobic acid was made according to the usual method. Average values for urinary ascrobic acid are given in Table I.

Table I will show that the enhanced excretion under chloretone can be suppressed when the animals are made either folic or pantothenic-acid deficient. The ascrobic-acid excretion could be strikingly stimulated again as soon as the missing vitamin was supplied.

TABLE I.

The urinary excretion of ascorbic acid (mg. in 24 hours) by chloretonized rats under different conditions.

Group number	Condition of animals	Number of rats	ASCORBIC ACID EXCRETION PER RAT:					
			1–3 days	4–6 days	7–10 days	11–13 days	14–16 days	17–19 days
I	Chloretonized ...	12	18·8	20·2	20·5	21·0	21·2	20·8
II(*a*)	Chloretonized—folic acid deficient ...	12	4·7	3·9	0·4	...	...	...
(*b*)	Chloretonized—pair-fed control with folic acid ...	12	16·9	19·2	20·8	21·2	21·0	20·5
(*c*)	Chloretonized—folic-acid deficient *plus* folic acid ...	12	...	...	...	4·5	15·8	20·2
III(*a*)	Chloretonized—pantothenic acid-deficient	12	3·8	1·2	0·5	...	...	...
(*b*)	Chlortonized—pair-fed control with pantothenic acid ...	12	18·5	20·5	21·2	21·0	20·8	21·5
(*c*)	Chloretonized pantothenic-acid deficient *plus* pantothenic acid	12	...	...	...	3·2	14·2	18·8

(*b*) *The effect of folic acid or pantothenic-acid deficiency on the storage of ascorbic acid in different tissues of rat under chloretone.*

All the rats used in the above experiment were sacrificed for the estimation of tissue ascorbic acid in order to note the effect of folic or pantothenic acid deficiency on its storage in different tissues. Liver, brain and kidney tissues were removed as soon as possible and made free from adhering blood clots. Tissue extracts for the determination of ascorbic acid were prepared in the usual manner. The results are given in Table II:—

TABLE II.

The tissue reserve of ascorbic acid (mg./100 g. *fresh tissue*) *in chloretonized rats under different conditions.*

Group number.	Condition of animal	ASCORBIC ACID:		
		Brain	Liver	Kidney
I	Chloretonized	47·3	32·4	24·5
II	Folic-acid deficient chloretonized	32·5	19·2	16·1
	Pair-fed control with folic acid	45·2	30·5	23·0
III	Pantothenic-acid deficient-chloretonized	30·5	18·4	15·0
	Pair-fed control with pantothenic acid	44·5	30·8	22·5

The results in Table II will show that both folic and pantothenic-acid deficiencies bring about a great reduction in the tissue ascorbic-acid content of rats treated with chloretone.

(*c*) *The effect of feeding ascorbic acid on the survival period of rats rendered deficient either in folic or pantothenic acid.*

The foregoing results indicate that the enhanced urinary excretion of ascorbic acid by the rat under chloretone can be completely suppressed and the tissue content reduced by rendering the animals deficient in either folic or pantothenic acid. So, it is apparent that along with the primary deficiency of folic acid there develops a secondary deficiency of ascorbic acid, the result being a double deficiency. So, it was of interest to observe the effect of feeding ascorbic acid on the survival period of folic or pantothenic deficient rats. The results are given in Table III:—

TABLE III.

The effect of feeding ascorbic acid on thes urvival period of either folic or pantothenic-acid deficient rats.

Group number	Number of rats	Condition of rat	Survival periods, days
I	12	Folic-acid deficient	10
	12	Folic-acid deficient *plus* 5 mg. ascorbic acid	16
II	12	Pantothenic-acid deficient	25
	12	Pantothenic-acid deficient *plus* 5 mg. ascorbic acid	32

Table III will show that there is a significant effect of ascorbic acid on the survival period of animals rendered folic or pantothenic-acid deficient.

(*d*) *The effect of feeding (i) glutamic acid and (ii) glycine on the urinary excretion of ascorbic acid by rats rendered folic acid-deficient and chloretonized.*

Glutamic acid is one of the components of aminopterin which is an analogue of folic acid, having 'NH_2' in place of 'OH' in position 4 of the molecule and glycine is known to be a good de-toxicating agent. So, these two amino acids were separately given to rats to counteract, if possible, the effect of aminopterin, so far as its suppressive action on the enhanced urinary excretion of ascorbic acid by the rat under chloretone was concerned. The average results are shown in Table IV.

It will appear from the results in Table IV that neither glutamic acid nor glycine has the power of counteracting the effect of aminopterin so far as the urinary excretion of ascorbic acid by rats is concerned. Glutamic acid, although a component of folic acid, cannot replace the latter in this regard.

TABLE IV.

The effect of feeding glutamic acid and glycine to folic-acid deficient animals on the urinary excretion of ascorbic acid by rats under chloretone.

Group number	Condition of rat	Number of rats	Ascorbic-acid excretion in mg./24 hours
I	Folic-acid deficient	12	0·4
II	Folic-acid deficient *plus* 20 mg. glutamic acid fed	12	0·5
III	Folic-acid deficient *plus* 20 mg. glycine fed	12	0·5

(*ii*) *Effect of deficiency of some B-vitamins on phosphorylated intermediary compounds present in the liver tissue of avitaminous rats.*

It has been previously reported (Ganguli, Roy and Guha, *Nature*, 1954, **174,** p. 511; *Technical Report*, *Scientific Advisory Board*, *Indian Council of Medical Research*, 1952, p. 59) that chloretone which stimulates ascrobic-acid synthesis in rats also depressed the formation of phosphate-esters present in the liver tissue of rats. Again, some B-vitamins, such as thiamin, riboflavin, folic and pantothenic acids have been found to be involved in the biosynthesis of ascorbic acid. The deficiency of those B-vitamins suppresses the synthesis of ascorbic acid stimulated by chloretone. So, the effect of their deficiency on the formation of phosphate-esters by the liver tissue of rats was studied. This work was carried out with non-chloretonized rats. The different phosphorylated compounds were estimated by the method of Umbreit *et al.* (Umbreit, Burris and Stauffer, ‘ Manometric Technique and Tissue Metabolism ’). The results are given in Table V.

Table V will show that single deficiency with respect to thiamin, riboflavin or folic acid increases the concentration of inorganic phosphate and decreases the different phosphate-esters present in the liver tissue of the avitaminous rats. This work will be followed up by work on chloretonized rats.

B. REGARDING THE PRECURSOR OF ASCORBIC ACID.

(*i*) *Study of several sugar acids as possible precursors of ascorbic acid.*—It was indicated in last year’s report that gluconic, glucuronic, saccharic and mucic acids could be converted *in vitro* into ascorbic acid by the tissues of the rat. There is, however, a marked differential behaviour in this respect between liver and brain tissues (Table VI).

TABLE V.

Effect of the single deficiency of thiamin, riboflavin and folic acid on the different phosphate-esters present in the liver tissue of avitaminous rats. Results are expressed in mg./100 g. wet tissue and represent average of 12 *animals.*

Group number	Condition of animal	In-organic -P	Total acid soluble P	Gluc.-1-P	Fruc.-6-P	Fruc.-1-6-P	Triose -P	Glyc. A-P	Pyr. -P	ATP
I	Thiamin deficient ...	36·6	79·7	18·9	22·5	19·5	14·6	17·4	6·2	0·21
	Pair-fed control ...	31·7	79·8	25·6	25·6	25·2	15·7	18·3	7·3	0·29
II	Riboflavin deficiency...	33·6	84·0	21·4	23·9	20·1	15·0	16·7	6·3	0·16
	Pair-fed control ...	29·9	80·9	23·9	26·1	25·0	16·8	19·5	7·1	0·28
III	Folic-acid deficient ...	37·2	89·8	17·6	19·3	19·8	13·3	15·7	6·1	0·20
	Pair-fed control ...	31·0	84·2	24·5	25·4	25·3	16·1	19·0	7·2	0·31

P=denotes phosphorus; Gluc. = glucose; Fruc. = fructose; Glyc. A = glyceric acid; Pyr. = pyruvic acid; ATP = adenosinetriphosphate.

TABLE VI.

Behaviour of washed brain and liver tissues of chloretonized rat towards utilization of different sugar acids for the synthesis of ascorbic acid (mg./100 g.).

Tissue	Buffer	Buffer +gluconate	Buffer +saccharate	Buffer +mucicate	Buffer +menthol	Buffer +menthyl glucuronate
Brain	14·5	24·9	22·3	22·9	14·6	21·3
Per cent increase ...	...	72·1	54·3	58·7	0·7	47·6
Liver	9·0	16·2	14·4	15·3	8·8	21·8
Per cent increase ...	...	79·5	59·8	70·0	—2·0	141·9

The fact that the conversion with brain tissue is the highest with gluconic acid and lowest with glucuronic acid, whereas glucuronic acid serves as the best precursor of ascorbic acid with the liver tissue appears to indicate that the mechanisms of biosynthesis of ascorbic acid with these two tissues are different in some important details. This is further supported by the preliminary observations on the differential behaviour of several inhibitors in regard to this biosynthesis depending on the nature of the tissue.

(*ii*) *Work with C*-14 *compounds.*—Previous *in vitro* and *in vivo* experiments with the tracer technique have indicated that pyruvic acid might be involved in the synthesis of ascorbic acid by the rat. Progress in the *in vitro* studies has also been reported which includes the effects of starvation, phosphate concentration of the incubating media, etc.

In vitro studies are now in progress with tracer compounds, viz., sodium pyruvate-2-C-14 and sodium acetate-1-C-14 as substrates with liver tissue from rats under different physiological conditions. Attempts will be made to separate and identify the intermediary compounds by chromatographic and radio-autographic techniques.

II. BIOCHEMICAL STUDIES ON NICOTINIC ACID.

In the last year's report determination of the bound nicotinic acid in pure bred strains of rice obtained from different parts of the country was made. This work has been continued during the year under review. Besides, the behaviour of bound nicotinic acid to cooking process has been studied.

(*a*) *Estimation of the bound and the total nicotinic acid.*—The varieties of raw paddy samples procured from different agricultural stations of the country were shelled by a P.S.G. sheller to remove the outer husk without disturbing the bran of the rice grain. The bound and the total nicotinic-acid contents of these samples were estimated by the method of Chaudhuri (4) using *p*-amino benzoic acid as a coupling agent. If the factor 'f' which is the ratio of the value for the total nicotinic acid to the value for the bound nicotinic acid of a sample of rice is about 3·75, it may be assumed that the sample contains all the nicotinic acid in the bound state as shown by Chaudhuri (*loc. cit.*). The results are given in Table VII:—

TABLE VII.

Bound and total nicotinic acid values of rice samples.

Serial number	Rice sample/strain number	NICOTINIC ACID (µG./G.)		'f' = $\frac{\text{Column (2)}}{\text{Column}}$
		Bound from (1)	Total (2)	
1	ADT-3	15·6	58·5	3·70
2	BAM-3	17·3	64·0	3·70
3	MTU-15	23·6	84·2	3·50
4	MTU-17	16·2	60·8	3·70
5	MTU-3	18·1	65·2	3·60
6	Kaimurali	19·0	57·3	3·00
7	Dhairal	20·3	67·7	3·30
8	Rupsail	18·2	66·4	3·65
9	Badshabhog	22·5	85·0	3·77
10	BAM-6	23·5	77·4	3·29
11	SLO-2	24·3	60·0	2·47
12	TKM-2	28·0	94·0	3·37
13	T-3	27·7	100·0	3·61
14	CO-13	20·0	70·4	3·52
15	TKM-1	19·4	73·6	3·79
16	GEB-1	17·5	64·0	3·66
17	CO-20	22·0	73·0	3·32
18	CO-3	19·0	66·0	3·42
19	CO-25	17·5	67·0	3·83
20	MTU-19	20·0	77·0	3·85

It can be seen from the values of 'f' in the Table VII that the major portion of the nicotinic acid is in the combined state.

(*b*) *Effect of cooking with distilled water on bound nicotinic acid.*—The rice samples used above were cooked separately with sufficient (about six times) distilled water (pH was about 6·6) for about 45 minutes. The gruel which was obtained after cooking was drained off and the bound and the total nicotinic acid values of the cooked rice samples were then determined by the method of Chaudhuri (*loc. cit.*). It was noted that the pH of the gruel was unchanged after cooking. The results obtained are given in Table VIII.

The results in Table VIII indicate that some of the nicotinic acid in the rice samples has been washed away with the gruel after cooking but almost all the nicotinic acid is found to be in the bound form as can be judged from the values of 'f'.

(*c*) *Nicotinic-acid values of the rice samples after cooking with tap-water.*—It was, then, thought desirable to study the effect of cooking of the above rice samples with tap-water, the pH of which was found to be about 8·4. The procedure followed here was the same as that adopted in the case of cooking with distilled water. It was observed that the pH of the gruel obtained after cooking did not change appreciably. The nicotinic-acid values obtained under different conditions were then determined by the usual method (*loc. cit.*) which are recorded in the Table IX.

TABLE VIII.

Nicotinic-acid values of the rice samples after cooking with distilled water.

Serial number	Rice sample/strain number	NICOTINIC ACID (μG./G.)		'f' = $\frac{\text{Column (2)}}{\text{Column (1)}}$
		Bound	Total	
1	ADT-3	8·40	30·30	3·60
2	BAM-3	11·0	41·80	3·80
3	MTU-15	10·0	38·6	3·80
4	MTU-17	11·5	39·2	3·40
5	MTU-3	10·0	36·0	3·60
6	Kaimurali	14·3	36·0	2·90
7	Dhairal	15·0	50·0	3·30
8	Rupsail	12	44·4	3·70

TABLE IX.

Nicotinic-acid values of the rice samples after cooking with tap-water.

Serial uumber	Rice sample/ strain number	NICOTINIC ACID (μG./G.)		'f' = $\frac{\text{Column (2)}}{\text{Column (1)}}$
		Bound	Total	
1	ADT-3	20·0	39·5	2·00
2	BAM-3	25·0	48·1	1·90
3	MTU-15	18·7	38·6	2·00
4	MTU-17	19·0	43·5	2·20
5	MTU-3	21·2	37·5	1·80
6	Kaimurali	17·6	37·0	2·10
7	Dhairal	18·0	37·0	2·20
8	Rupsail	16·0	32·8	2·05

It may be noticed from the values of 'f' in Table IX that the tap-water having a pH of about 8·4 used for cooking the rice samples decomposed a portion of the bound nicotinic acid resulting in the liberation of free nicotinic acid.

III. STUDIES ON METABOLISM OF IRON DURING THE EMBRYONIC DEVELOPMENT OF THE FERTILE HEN'S EGG.

Synopsis of the work.

(1) The effects of phosphate, glucose and sodium chloride on the oxygen equilibrium curve of chick hæmoglobin were observed. These tended to make the oxygen equilibrium curve hyperbolar. Cysteine added to intact red blood corpuscles also renders the oxygen equilibrium curve slightly hyperbolar.

(2) The behaviour of alkali-resistant hæmoglobin was studied spectrophotometrically.

(3) The fractionation of iron-containing proteins of the livers of the chicks during the early development was undertaken.

(4) The release of iron from the egg-yolk proteins depended on the reducing capacity of the reagents used.

(1) *Oxygen equilibrium curves of chick hæmoglobin under different conditions.*

Effect of phosphate concentration.—The effect of varying the phosphate concentration on the oxygen equilibrium curve of adult chick hæmoglobin was studied. The hæmoglobin solution was adjusted to different phosphate concentrations by adding to it 1·0 molar phosphate buffer (NaH_2PO_4: Na_2HPO_4—pH 7·4) in different proportions. The oxygen equilibrium curves of these solutions were then determined (Fig. 1):—

FIGURE 1.

Effect of phosphate concentration on oxygen equilibrium curve.

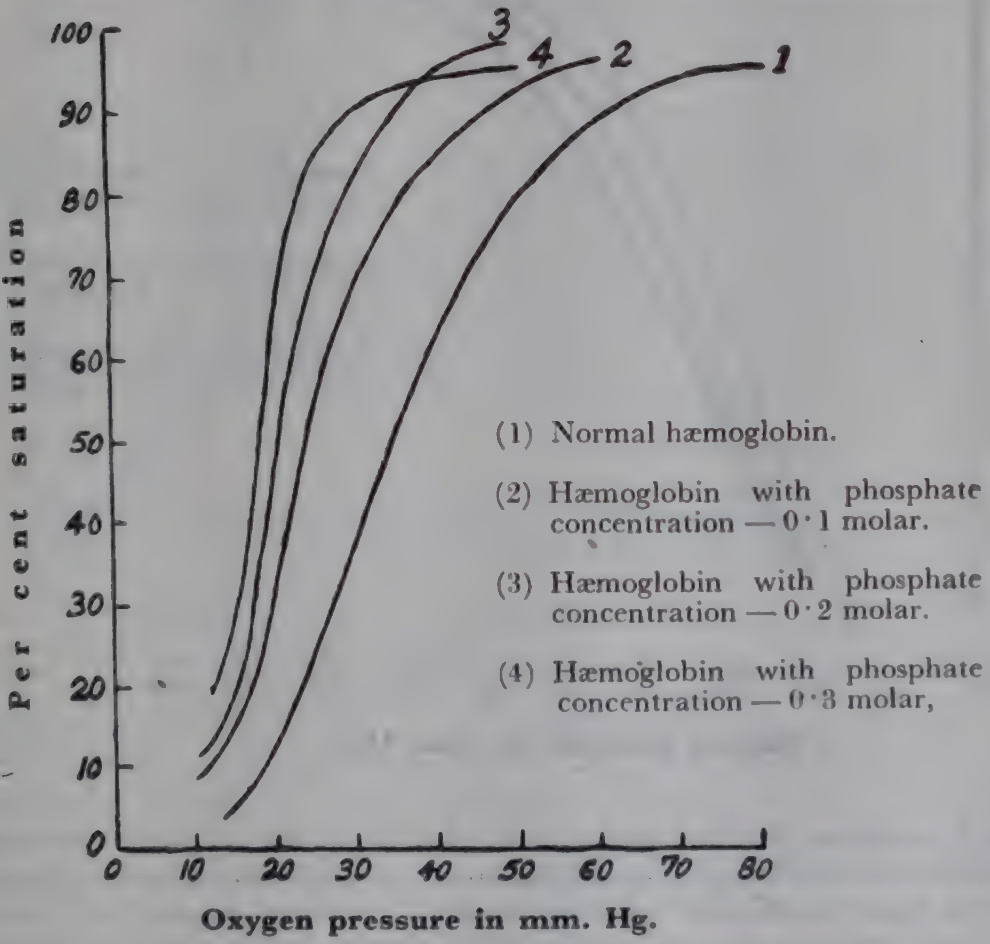


Fig. 1 shows that with the increase in phosphate concentration of the adult chick hæmoglobin solution, the oxygen equilibrium curve tends to be hyperbolar.

Effect of glucose.—Wyman (*Jour Biol. Chem.*, 1953, **203,** p. 81) reported that when the adult and fœtal human hæmoglobins were dialysed against 0·4 molar phosphate buffer containing 1·0 molar glucose, the oxygen equilibrium curves became similar in shape, while without such treatment they were different. It was reported last year that the oxygen equilibrium curves of hæmoglobin from adult and newly hatched chicks were not similar when dialysed likewise, although the curve of the dialysed hæmoglobin solution in each case became more sigmoid-shaped than that of the undialysed solution. In this dialysis, glucose had entered the hæmoglobin solution. It was considered desirable to study the effect of known concentration of glucose on the oxygen equilibrium curve. Four c.c. of the hæmoglobin solution were taken and the desired glucose concentration in the hæmoglobin solution was obtained by adding different proportions of 2 M glucose to it. The curves are shown in Fig. 2:—

FIGURE 2.

Effect of glucose on oxygen equilibrium curve.

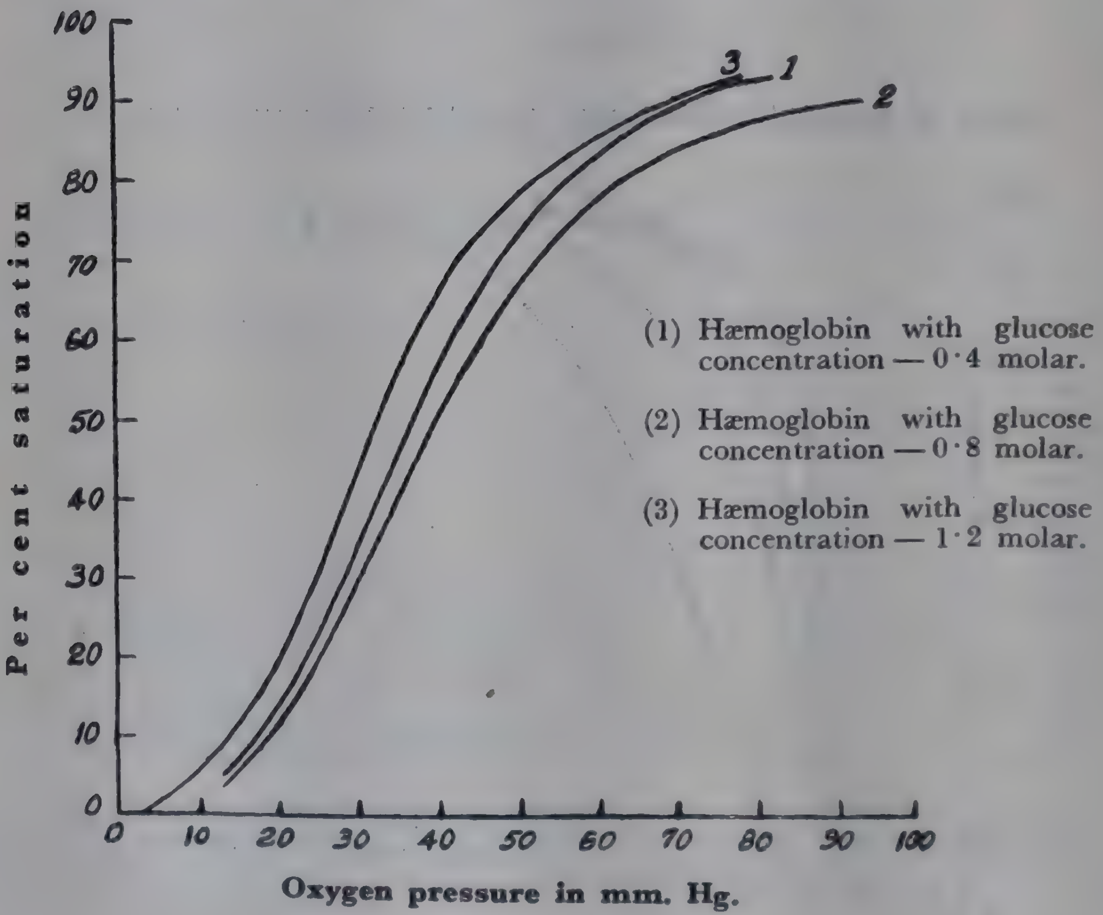


Fig. 2 confirms that the presence of glucose in the hæmoglobin solution, generally renders the oxygen equilibrium curve sigmoid-shaped. Curiously the highest concentration of glucose used (1·2 molar) appears to make the oxygen equilibrium curve of adult chick hæmoglobin slightly hyperbolar in comparison with the curves obtained with lower concentrations of glucose, viz., 0·4 and 0·8 molar.

Effect of dialysis on the oxygen equilibrium curve of hæmoglobin.—The molarity of the phosphate buffer in the hæmoglobin solution was adjusted by adding different amounts of molar phosphate buffer (Na_2HPO_4: NaH_2PO_4—pH 7·4) to the hæmaglobin solution. A volume (10 c.c.) of such a hæmoglobin solution was dialysed for 16 to 18 hours at 5°C. in a refrigerator againt 100 c.c. of phosphate buffer of different molarities. The dialysed and undialysed solutions were used for determination of oxygen equilibrium (Fig. 3):—

FIGURE 3.

Effect of dialysis on oxygen equilibrium curve.

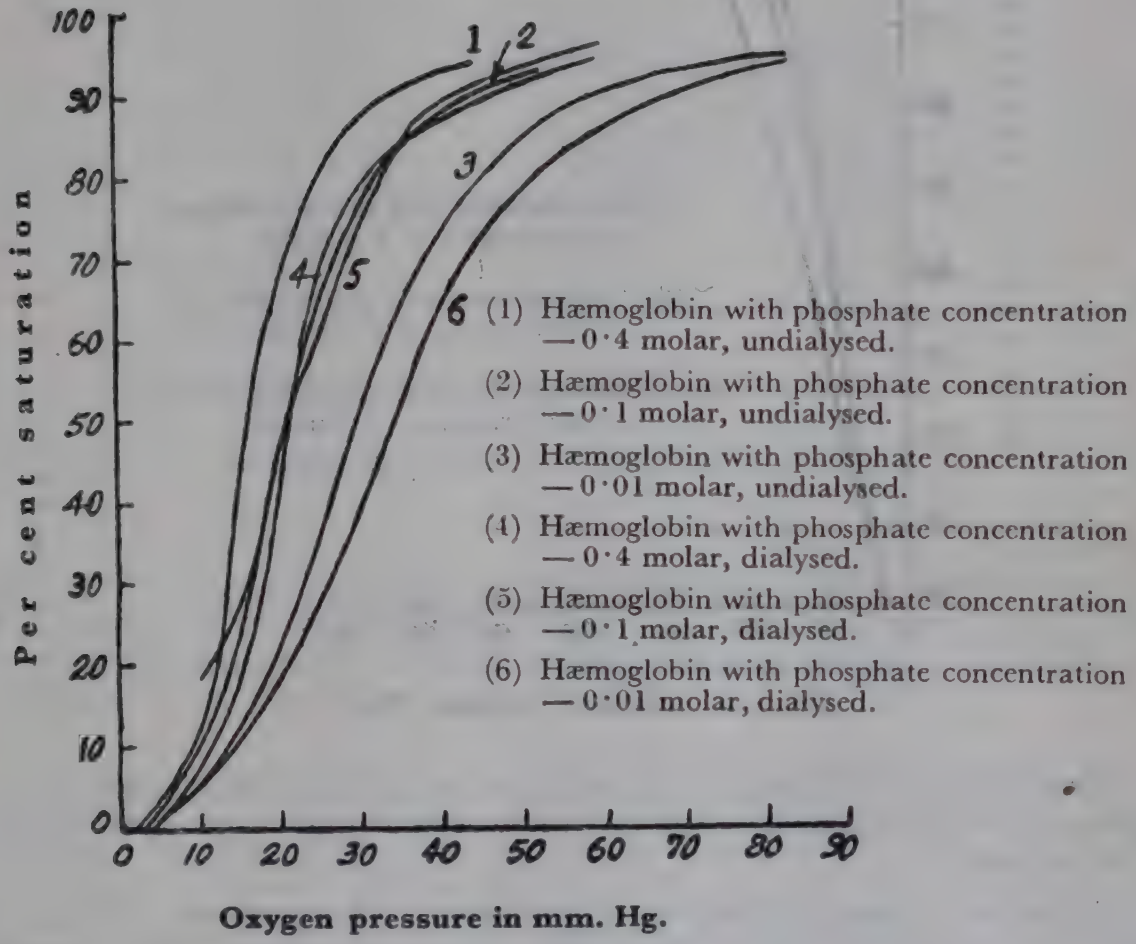
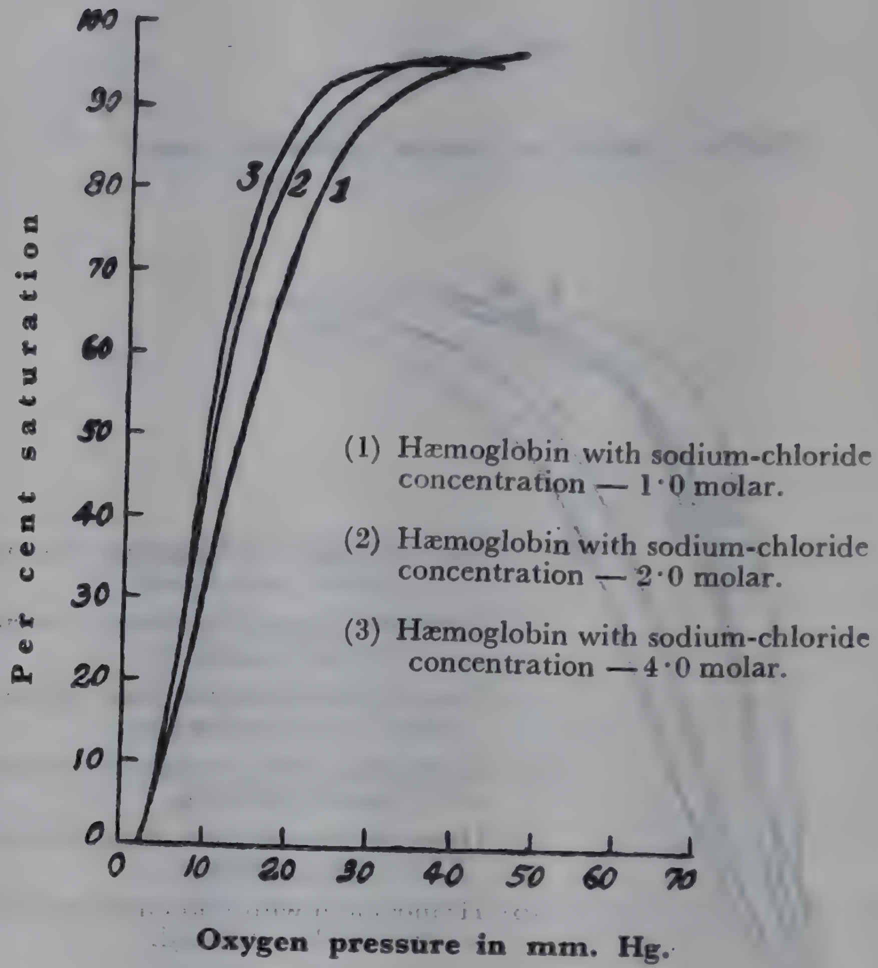


Fig. 3 shows that in the case of undialysed hæmoglobin, the oxygen equilibrium curve shifts increasingly towards the left with the increase of phosphate concentration. With the dialysed hæmoglobin the same effect is observed except in the case in which the solution was dialysed against 0·1 molar phosphate. This has to be confirmed by further experiments.

Effect of sodium chloride on the oxygen equilibrium curve.—It was reported last year that in strong urea solution, the adult chick hæmoglobin gives hyperbolic oxygen equilibrium curve. The same nature of the curve has been obtained by treating Hb with strong sodium-chloride solution. In 6 molar sodium chloride solution, the hæmoglobin solution became viscous, so that further photometric measurements could not be taken. The desired sodium chloride concentration in the hæmoglobin solution was obtained by adding solid sodium chloride to the solution and the oxygen equilibrium curve of the solution was then determined (Fig. 4).

FIGURE 4.

Effect of sodium chloride on oxygen equilibrium curve.



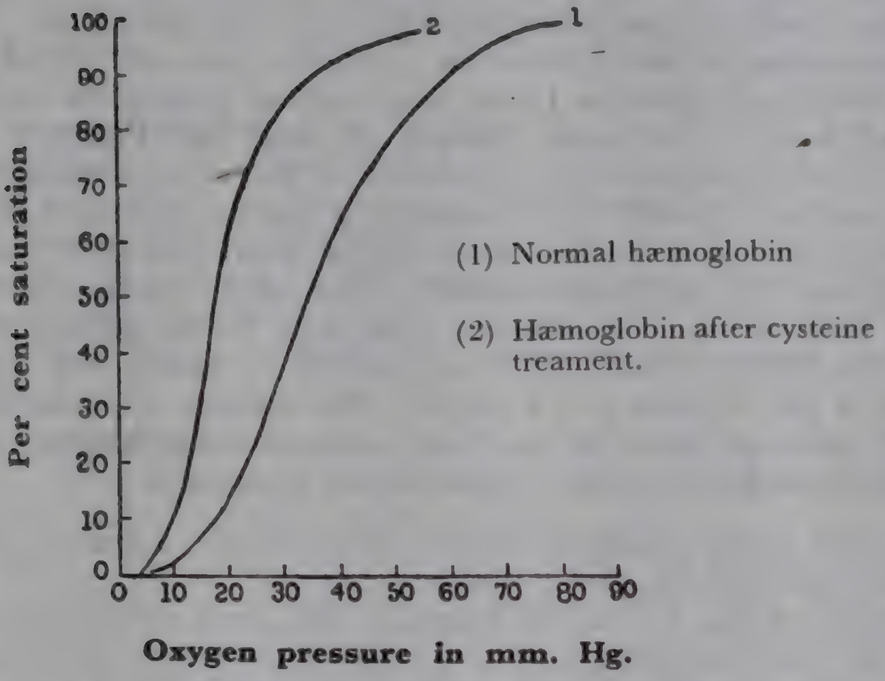
From Fig. 4 it appears that with increasing sodium-chloride concentration in the hæmoglobin solution, the oxygen equilibrium curve tends to be hyperbolar.

Effect of cysteine on hæmoglobin of intact cell.—To a mixture of 4 c.c. of red blood corpuscles and 4 c.c. of 0·1 molar phosphate buffer, cysteine hydrochloride (13·69 mg.) was added, so that this suspension contained 12·78 g. hæmoglobin per 100 c.c. and cysteine hydrochloride 0·171 g. per 100 c.c. The oxidation-reduction potential of this suspension, as measured by Phillips pH meter with a platinum and a calomel electrode, is 252 m.v. at 34°C. The suspension was kept for half-an-hour and then washed with normal saline. The packed cell-volume of the red blood corpuscles was determined. The red blood corpuscles were lysed with twice the volume of water and 0·4 volume of toluene. The pH of the solution was measured by glass-electrode and found to be 6·6 at 34°C. The concentration of hæmoglobin in the final solution was 5·45 g./100 c.c. (estimated as carbonmonoxyhæmoglobin). The oxygen equilibrium curve of this solution was determined (Fig. 5).

Fig. 5 shows that cysteine shifts the curve to the left. Incidentally, the red blood corpuscles were stained with Giemsa stain and it was observed that the cytoplasm portions were damaged apparently as an effect of cysteine.

FIGURE 5.

Effect of cysteine on hemoglobin of intact cell.



(2) *The behaviour of alkali-resistant hæmoglobin.*—This work was undertaken to throw more light on the nature of the alkali-resistant hæmoglobin which is predominant in embryonic chick's blood and which remains in a considerable amount even up to the 15th day after hatching.

That there are differences in the resistance of fœtal and adult blood to denaturation by alkalis and acids was demonstrated by Körber (*Inaug. Dissertation*, Dorpat, 1866) and then by von Krüger (*Z. Biol.*, 1888, **34**, p. 318) and Haurowitz (*Hoppe-Seyl Z.*, 1930, p. 186). It was shown by Haurowitz (*Z. Physiol. Chem.*, 1929, **183**, p. 78; Brinkman, (*Jour. Physiol.*, 1934, **80,** p. 337) that both types of hæmoglobin—alkali-resistant and alkali-labile, may be present in the same blood. The alkali denaturation was quantitatively studied by Singer, Chernoff and Singer (*Blood*, 1951, **6,** p. 413). 0·1 c.c. of the hæmoglobin solution from a seven-day old chick which generally contained about 10 g. hæmoglobin/100 c.c. was added to 1·6 c.c. of N/12 NaOH, pH 12·7 at 20°C., and the portion which was denatured within one minute after the addition of hæmoglobin to the alkaline solution was termed as alkali-labile portion. The remaining portion of the original hæmoglobin solution which was obtained after the addition of ammonium-sulphate solution containing hydrochloric acid after one minute interval was termed as the alkali-resistant portion. The ammonium-sulphate solution containing hydrochloric acid neutralized the alkali present and also effected the precipitation of the denatured portein. The solution was filtered and the optical density of the filtrate was determined in a Beckmann spectrophotometer at 540 m.μ. The hæmoglobin solution was obtained after washing the red blood corpuscles four times with normal saline in the cold. Then the cells were lysed with water of a volume which was twice the volume of the packed cells as measured by centrifuging the cells at 2,500 r.p.m. for 15 minutes at 2° C. The lysed solution was centrifuged twice at 3,500 r.p.m. for half an hour each time. The hæmoglobin content of the solution was determined spectrophotometrically. 0·1 c.c. of the hæmoglobin solution and excess of sodium dithionite was added to it and carbon monoxide was passed through the solution. The optical density of the carbon-monoxyhæmoglobin was determined at 540 m.μ., and the concentration of hæmoglobin, in g. per litre was calculated from the absorption co-efficient, dilution factor and optical density of the solution (Lemberg and Legge, 'Hæmatin compounds and Bile Pigments'). The study of the spectrophotometric behaviour of the alkali-resistant part of hæmoglobin was then studied. The absorption spectra of the alkali-resistant portions were determined in the visible region from 450 to 625 m.μ. in a Beckmann Quartz DU spectrophotometer. The alkali-resistant hæmoglobin solution was collected in a graduated tube and molar phosphate buffer of pH 7·4 (Na_2HPO_4—NaH_2PO_4) was added to bring the phosphate concentration of the solution to 0·1 molar. The spectra of oxyhæmoglobin, reduced with sodium dithionite and of carbon-monoxyhæmoglobin were then determined under the same experimental condition.

In the case of the dialysed portion, 5 c.c. of the solution containing alkali-resistant hæmoglobin were dialysed against 50 c.c. of 0·1 molar phosphate buffer of pH 7·4 for 48 hours at 5° C. in a refrigerator. As in the case of the non-dialysed portion, the absorption spectra of oxyhæmoglobin, hæmoglobin reduced with sodium dithionite, and carbonmonoxyhæmoglobin were obtained. The peaks of absorption spectra thus obtained are given in Table X:—

TABLE X.

The behaviour of alkali-resistant hæmoglobin.

		7-DAY OLD CHICK HÆMOGLOBIN:					
		Normal		Alkali-resistant			
		Undialysed		Undialysed		Dialysed	
HbO_2	Minima	508	562	512	562	518	565
	Maxima	540	578	540	580	538	580
Reduced Hb	Minima	483		486		486	
	Maxima	554		558		556	
HbCO	Minima	498	556	498	556	498	556
	Maxima	540	570	540	570	540	570

Table X shows that there are marked differences in the absorption spectra of the hæmoglobin of seven-day old chicks as determined with the whole hæmoglobin solution as after the separation of alkali-denatured protein. This is a further indication of the existence of the two types of hæmoglobin. That the treatment of alkali does not appreciably alter the character of the alkali-resistant hæmoglobin has been shown from the solubility experiments by Derrien, Laurent and Borgomans (*C. R. Soc. Biol.*, 1955, **149,** p. 137).

(3) *Studies on Ferritin.*—It was of interest to study the metabolism of iron in the liver of the chick after different periods after hatching in order to get an idea of the changes in this metabolism with the growth of the chick.

After hatching, the iron deposited in the liver of the chick is used for the production of heme-pigments. It was shown by Granick (*Chem. Rev.*, 1946, **38,** p. 379) that storage iron in the liver may be classified as ferritin and hæmosiderin and these are the compounds which are metabolized according to the necessity of the hæmopoietic system. Attempts have been made to fractionate the different compounds and to study the metabolism of these compounds in the chick liver one and seven days after hatching.

Experimental procedure.—A sample of 2 g. to 5 g. of chick liver was taken and washed free of blood with 0·9 per cent sodium-chloride solution. A certain amount was taken for dry weight analysis and was then analysed for total iron. The liver was cut into small pieces and ground in a mortar contained in an ice-bath and two volumes of cold water were added. The mortar was washed with 0·35 volumes of water. The suspension was kept for ten minutes at room temperature and centrifuged at 5° C. at 3,500 r.p.m. for half an hour. The supernatant liquid was removed by a fine glass jet and the sediment was again mixed thoroughly with two volumes of water and re-centrifuged for half an hour at 3,500 r.p.m. The two supernatant fluids were mixed and were ready for the estimation of ferritin. The sediment, left after these two centrifugations, was digested with concentrated sulphuric acid and perchloric acid and the iron was determined by developing colour with thioglycolic acid and ammonia in the Lumetron.

The supernatant solution generally contains certain amounts of hæme-proteins which may be estimated by the pyridine-hæmochromogen method of Flink and Watson (*Jour. Biol. Chem.*, 1942, **146,** p. 171) at wavelengths, 500 and 555 m.μ This gives the hæme-iron present in the pooled extract and the differer[illegible] the hæme-iron and the total iron in this solution gives the iron extracta[illegible]e by water only which is a measure of the ferritin iron present.

In the next step, the supernatant was taken in 100 c.c. beaker and heated to 75°C. on a boiling water-bath. Most of the hæme-proteins coagulated and the solution was centrifuged at 5° C. for half an hour at 3,500 r.p.m. The heat-coagulum was again mixed with the same volume of water and re-centrifuged. After pooling the extract, the heat-coagulum was taken for the analysis of total iron.

From an aliquot of the supernatant fluid after heat treatment, the total iron was estimated after sulphuric acid and perchloric acid digestion and the nitrogen was estimated by the method of Ma and Zuazaga (*Ind. Eng. Chem.*, 1942, **14,** p. 280).

The pooled heat-supernatant fluid was treated with ammonium sulphate (35 g./100 c.c.) and the precipitate formed was allowed to settle and kept overnight in a refrigerator and spun down at 5° C. at 3,500 r.p.m. for half an hour. The supernatant liquid was then siphoned off and discarded. The precipitate so obtained was dissolved in 5 c.c. to 10 c.c. of water and the total iron of the solution was estimated as before.

Effect of heat on the precipitation of proteins.—It was mentioned by Granick (*Chem. Rev.*, 1946, **38,** p. 379) that by heating liver extracts for five minutes at 80°C., most of the proteins including hæme-proteins would precipitate, while ferritin would be in solution.

It was, however, of interest to study the effects of heating for different periods, *viz.*, one, three, five and ten minutes at 75° C. The iron and nitrogen of the heat-supernatant fluid and the heat-coagulum obtained under these conditions were, therefore, estimated. The material available with one day old chick's liver was not, however, sufficient for these experiments. These were, therefore, carried out with the liver of seven-day old chicks. These results show that the iron content of the heat-supernatant solution first decreases and then increases with the period of heating, while the nitrogen content of the supernatant decreases steadily. Further studies on the metabolism of ferroproteins at different ages of the chick are in progress.

TABLE XI.

Fraction of chick liver ferro-proteins.

Chick	Liver taken, g.	Water extract total iron, mg.	Residue total iron, mg.	Heat super-natant total iron, mg.	Heat coa-gulum total iron, mg.	Ammonium sulphate precipitat total iron, mg.
One day old	3·7	0·6	1·005	0·415	·15	·125
Seventh day old	2·5	0·570	0·1875	0·38	0·175	0·14

(4) *Effect of change of oxidation-reduction potential on the release of iron from egg-yolk protein.*—That the iron in incubated fertile or fertile hens' eggs is present as a complex of ferric iron and protein is known. By simple trichloro-acetic acid extraction this cannot be liberated and it was observed by Tompsett that if the egg-yolk be treated with reagents, such as sodium dithionite, sodium pyrophosphate, or thiolactic acid, iron is liberated and can be estimated (Tompsett, *Biochem. Jour.*, 1935, **29,** p. 480). It has now been shown by Ramsay (*Biochem. Jour.*, 1951, **49,** p. 494) that in the white Leghorn hens' eggs, the non-hæmatin iron changes to hæmatin iron with incubation. The conclusion is based on the simple technique in differential liberation of iron from the solution containing egg-yolk protein. The synthesis of hæme-proteins takes place within the eggs and the liberation of iron for such purposes depends on certain factors, such as pH, oxidation potential of the system, and also the requirement for iron by the hæmopoietic system. The present investigation was undertaken to study the effect of change in the oxidation-reduction potential on the release of iron from its complex combination with protein in egg-yolk.

The yolk of one infertile egg was taken and well mixed with water and the volume was made up to 100 c.c. A definite amount of this solution was taken and treated separately with sodium dithionite, sodium pyrophosphate and thiolacetic acid. Ten-c.c. portions of the resulting solution were added at definite intervals of time (5, 10 and 15 minutes) to 10 c.c. of 20 per cent trichloro-acetic acid and the supernations was siphoned off and the precipitate was washed with 5 c.c. of five per cent trichloro-acetic acid. To the filtrate and washing, six drops of thioglycolic acid and 4 c.c. of concentrated ammonia were added. The precipitate, if formed, was filtered and the colour density was measured in the Lumetron.

The oxidation-reduction potential of the system containing egg-yolk and the reagents and the reagents used as measured by the Phillips pH meter was also estimated at every stage in each case to study the correlation between the redox potential and the release of iron.

The result shows that the liberation of iron from its protein complex depends on the reducing capacity of the reagent used. With the increase of the reduction potential of the system, the amount of iron liberated from its protein complex increases.

TABLE XII.

A. *Effect of sodium dithionite.*

30 C.C. OF EGG-YOLK SOLUTON+1·3 G. OF SODIUM DITHIONITE:-

Time after the addition of sodium dithionite, minute	Millivolts	Iron, mg./10 c.c.
5	—67	0·0162
10	—71	0·0202
15	—71	0·0366

30 C.C. OF EGG-YOLK SOLUTION+1·3 G. OF SODIUM DITHIONITE.

Time after the addition of sodium dithionite, minute	Millivolts	Iron, mg./10 c.c.
5	—77	0·026
10	—86	0·028
20	—83	0·028

B. *Effect of sodium pyrophosphate.*

30 C.C. OF EGG-YOLK AND 2 C.C. OF 20 PER CENT SODIUM PYROPHOSPHATE:

Time after the addition of sodium pyrophosphate, minute	Millivolts	Iron, mg./10 c.c.
5	—31·5	0·0101
10	—33·5	0·0201
15	—33·0	0·0200

C. *Effect of thiolacetic acid.*

30 C.C. OF EGG-YOLK SOLUTION + 0·6 C.C. OF THIOLAÇETIC ACID.		
Time after the addition of thiolacetic acid, minute	Millivolts	Iron, mg./10 c.c.
5	—211	0·028
10	—221·5	0·028
15	—229	0·0282

IV. EFFECT OF CANNING AND STORAGE ON THE NUTRITIVE VALUE OF SOME COMMON INDIAN VEGETABLES.

Synopsis of the work.—Effect of different canning operations and of storage on some nutrients in peas (*Pisum sativum*) has been studied. During blanching the average losses of ascorbic acid, thiamin, riboflavin and nicotinic acid amount to about 24, 15, 28 and 11 per cent, respectively.

During autoclaving the blanched samples, the loss of ascorbic acid, thiamin and nicotinic acid come to about 48, 17 and 15 per cent, respectively, of what remained after blanching. No loss of riboflavin occurs during autoclaving.

During storage for three months the average loss of ascorbic acid is 74 per cent, of thiamin 20 per cent and of nicotinic acid about 30 per cent of the vitamins that remained after autoclaving. There is, however, an increase in riboflavin during storage, the average increase is 117 per cent of the riboflavin content of the blanched samples.

An increase in ascorbic acid in cauliflower during blanching was reported last year. This was further investigated this year. An increase in ascorbic acid during blanching was observed in 45 per cent of the samples of cauliflowers of different sizes, varieties, colours, textures, seasons and maturity and from different localities.

(1) Peas (*Pisum sativum*).—The samples of peas were purchased from the local market and depodded by hand. About 4 kg. of the samples were brought to the laboratory and the immature and overmature grains were rejected by hand-picking. The sample was then floated in a tall glass-cylinder. Only the grains that settled at the bottom were taken for experimental studies. The sample was dried by a towel and representative portions were taken for different estimations. Their average values per 100 g. are given below:—

Moisture ...	...	73·6 g.
Ascorbic acid	...	15·8 mg.
Thiamin ...	...	418·0 μg.
Riboflavin	...	150·3 μg.
Nicotinic acid	...	3·1 mg.

About 3 kg. of the sample were blanched for five minutes at 85°C. in three litres of tap-water in an aluminium vessel. The blanched sample was cooled to room temperature by a spray of water.

Lots of 200 g. of blanched samples were weighed into cans of size 301 × 309 and covered with 1·5 per cent sodium chloride solution leaving a head-space of 1/8″. Filled cans were exhausted by placing them in a water-bath till the temperature of the contents reached 80°C. The cans were then sealed by double seaming. The sealed cans were autoclaved at 10-lb. pressure for 25 minutes, cooled to room temperature by a spray of water and stored in the laboratory. One can was opened immediately after processing and others after seven days, fifteen days, one month, two months, and three months and drained for five minutes in a large colander. Weights of solids and volumes of liquid portions were recorded. The ascorbic acid, thiamin, riboflavin and nicotinic acid contents of both the solid and liquid portions were determined. The moisture content of the solid portions was also estimated.

Ascorbic acid determinations were made by using 2:6 dichlorophenol indophenol dye as recommended by the Association of Vitamin Chemists, Inc. (1947), thiamin by the method of Harris and Wang (*Biochem. Jour.*, 1941, **35,** p. 1050) riboflavin by the method recommended by the Association of Vitamin Chemists, Inc. (1947) and nicotinic acid by the method of Chaudhuri (*Ind. Jour. Med. Res.*, 1951, **39,** p. 491) in a photo-electric apparatus. The results represent the mean values of six samples studied.

Effect of blanching.—During blanching at 85°C. for five minutes ascorbic acid is lost up to an average of 24 per cent, thiamin 15 per cent, riboflavin 28 per cent and nicotinic acid 11 per cent.

Effect of autoclaving the blanched samples.—The blanched samples were weighed into cans and autoclaved at 10-lb. pressure for 25 minutes. The changes in ascorbic acid, thiamin, riboflavin and nicotinic acid have been determined. The distribution of the vitamins in the solid and liquid portions of the can contents was also investigated.

During autoclaving the blanched samples, ascorbic acid is lost to an average of 48 per cent of what remained after blanching and the remaining ascorbic acid is found distributed in the solid and liquid portions of the can contents in the ratio of 3 : 2.

The loss of thiamin during autoclaving amounts to 17 per cent of the vitamin that remained after blanching. The vitamin is distributed in the solid and liquid portions of the can contents in the ratio of 2 : 1.

There is practically no loss of riboflavin during autoclaving of peas. The vitamin is distributed in the ratio of 3:1 in the solid and liquid portions of the can contents.

About 15 per cent of the nicotinic acid that remained after blanching is lost during autoclaving. The vitamin is distributed in the solid and liquid portions in the ratio of 2 : 1.

Effect of storage on the canned products.—The canned samples were stored in the laboratory (temperature varying from 23°C. to 40°C.) in cans and the changes in ascorbic acid, thiamin, riboflavin and nicotinic acid were determined after seven days, fifteen days, one month, two months and three months. The distribution of the vitamins in the solid and liquid portions of the can contents was also noted.

About 22 per cent of ascorbic acid, that remained after autoclaving, is lost during the first week of storage and 32, 43, 67 and 74 per cent, is lost during storage for 15 days, one month, two months and three months, respectively. The distribution of the vitamin in the solid and liquid portions of the can contents during storage up to one month is roughly in the ratio of 3 : 2.

The loss of thiamin during storage for three months is about 20 per cent of what remained after autoclaving the product. The loss increases progressively during storage—12 per cent during the first week, 15, 18, 18·9 and 20 per cent during storage for 15 days, one month, two months and three months, respectively. The vitamin is distributed in the solid and liquid portions of the can contents roughly in the ratio of 3 : 2 up to 3 months' storage.

During storage for three months, the riboflavin content of green peas increases to a considerable extent, 117 per cent of what remained after autoclaving. The increase is progressive with the duration of storage, six per cent during the first week, 30, 61, 100 and 117 per cent during storage for fifteen days, one month, two months and three months, respectively. This increase is observed in both the solid and liquid portions of the can contents. The distribution of the vitamin between the solid and liquid portions during the whole period of storage is roughly in the ratio of 4 : 1. Brenner *et al.* (*Food Technol.*, 1948, **2,** p. 207) observed an increase of 200 per cent of riboflavin in peas during storage for 19 months. Murray (*Food Res.*, 1948, **13,** p. 397) also observed an increase in riboflavin in peas on long cooking.

About 14 per cent of nicotinic acid that remained after autoclaving was lost during 1st week's storage. During storage for 15 days, one month, two months and three months the respective losses were 24, 26, 27 and 32 per cent.

(2) Cauliflower (*Brassica olerecebotrytes*).—The effect of different canning operations and of storage on the ascorbic acid content of cauliflower was reported last year. It was observed that there might be an increase of ascorbic acid in cauliflower during blanching. To confirm this observation a detailed programme of studies was pursued.

Cauliflower was purchased from the local market. After washing and cleaning it was cut into pieces with a stainless steel knife and 200 g. of the sample were blanched in 600 c.c. distilled water in a beaker at 82°C. for four minutes. The blanched sample was spread on a circular enamelled dish (of 20-inch diameter) for 30 minutes. Ascorbic acid was estimated in the raw and blanched samples and in the blanching liquor. A portion of the raw sample (shredded) was kept in the laboratory (at room temperature varying from 19°C. to 30°C.) and the vitamin was estimated after three hours.

The results are given in Table XIII.

Table XIII shows that there is an increase of ascorbic acid during blanching in some of the samples studied. It is not yet clear on what characteristics of the cauliflower this rise in ascorbic acid depends.

The shredded raw samples of cauliflower were kept in the laboratory (at 19°C. to 30°C.) and ascorbic acid was estimated after three hours. From Table XIII it appears that the ascorbic acid content of shredded raw samples of cauliflower increases in some cases, on standing for three hours. Prokoschev (*Biochimia*, 1944, **9,** p. 36) observed increase of ascorbic acid at the cut surfaces of potato. The reasons for the increase in ascorbic acid content in some cases will be investigated.

TABLE XIII.

Ascorbic acid (mg.) per sample (200 *g.*).

Date	Raw tissue	BLANCHED			Per cent change in total ascorbic acid on blanching	Raw tissue after 3 hours (at room temperature)*	Per cent change in ascorbic acid on standing at room temperature*
		Tissue	Liquor	Total			
29–11–54	165·4	111·0	33·1	144·1	—12·8	165·4	nil
2–12–54	147·6	101·7	49·6	151·3	+2·5	138·4	—6·2
15–12–54	205·2	163·0	26·0	189·0	—8·0	208·6	+1·6
20–12–54	142·8	101·2	36·3	137·5	—5·2	147·2	+3·0
22–12–54	173·0	138·1	34·1	172·2	—0·5	170·8	—1·2
10–1–55	203·4	185·4	25·7	211·1	+3·6	210·8	+3·6
11–1–55	194·8	153·8	31·7	185·5	—4·7	203·4	+4·4
13–1–55	175·6	167·1	28·6	195·7	+10·2	170·6	—2·8
16–1–55	129·0	113·8	20·4	134·2	+4·0	136·0	+5·4
18–1–55	106·2	81·0	18·8	99·8	—5·8	102·4	—3·3
20–1–55	156·8	117·1	13·9	131·0	—18·8	158·2	+0·8
24–1–55	128·2	104·0	24·5	128·5	nil	129·0	+0·6
31–1–55	122·0	106·0	13·3	119·3	—2·3	119·6	—2·0
9–2–55	145·6	126·0	34·9	160·9	+9·0	168·6	+16·7
16–2–55	181·8	192·8	24·4	217·2	+19·4	203·6	+11·9
17–2–55	215·6	186·4	22·3	208·7	—3·2	222·2	+3·0
21–2–55	255·8	265·0	28·7	293·7	+14·8	268·2	+4·8
23–2–55	231·4	222·0	22·0	244·0	+5·4	220·0	—4·9
26–2–55	233·0	195·3	24·4	219·7	—5·7	240·0	+3·0
28–2–55	233·0	221·2	24·8	246·0	+5·5	272·6	+17·0
7–3–55	154·8	131·8	24·0	155·8	+0·4	176·4	+7·0
11–3–55	135·2	105·4	16·2	121·6	—10·1	146·4	+7·7

*Room temperature varied from 19° C. to 30° C.

V. STUDIES ON THE LOSS OF NUTRIENTS OF PURE-BRED STRAINS OF RICE DURING COOKING.

Synopsis of the work.—Studies on the loss of nutrients of two pure-bred strains of rice were reported last year. In order to complete this work, three more pure-bred samples of rice have been investigated. As had been observed before there is significant loss of some nutrients during washing the rice prior to cooking. It has also been confirmed that there is practically no loss during cooking with just sufficient water, which is usually 3 to 3·5 times (w/v) of the rice. But cooking with excess water and throwing the gruel cause considerable loss of nutrients and the loss is more when the rice is cooked with more water.

The paddy samples (raw, sun-dried), ADT-3 (Aduturai), T. 3 (Nagina, U.P.) and Charnock (West Bengal) were obtained from different States and were shelled in a P. S. G. huller at a standard speed of 90 strokes per minute. The shelled rice thus obtained had its husk only removed and was taken for cooking.

200 g. of rice were washed three times with 200 c.c. of distilled water and the washed rice was cooked in an aluminium pot with just sufficient water, six volumes (w/v) of water and eight volumes (w/v) of water till the rice was sufficiently soft for consumption. The gruel was drained for a definite period of time (ten minutes). The loss of thiamin, riboflavin, nicotinic acid and of calcium, phosphorus, phytin phosphorus, iron and available iron in wash water and in gruel was estimated. The retention of these nutrients in the cooked rice was also determined.

Results of the three varieties of rice are given in Tables XIV, XV, XVI, XVII, XVIII and XIX:—

TABLE XIV.

Per cent loss of ADT-3 during washing and cooking.

	In washing	IN GRUEL OF RICE COOKED WITH:	
		6 volumes of water	8 volumes of water
Ash	11·3	13·7	16·7
Nitrogen	2·5	4·0	7·4
Thiamin	3·9	23·5	31·0
Riboflavin	25·0	37·5	46·7
Nicotinic acid	10·2	32·9	36·3
Calcium	8·8	16·5	24·7
Phosphorus	6·6	13·9	16·8
Phytin phosphorus	4·7	13·5	16·7
Iron	22·2	22·2	30·0
Available iron	24·0	20·0	22·0

The cooking time was 30 minutes, 33 minutes and 34 minutes with just sufficient water (700 c.c.), six volumes and eight volumes of water, respectively. The respective weights of the cooked rice were 650 g., 648 g., and 647 g. The moisture contents of the corresponding cooked rice samples were 73·5, 72·6 and 74·9 per cent, respectively. Table XIV shows the loss during washing and cooking.

The percentage retention of the nutrients in cooked rice is shown in Table XV:—

TABLE XV.

Percentage retention of nutrients in cooked rice (ADT-3).

	RICE COOKED WITH:		
	Just sufficient water	Six volumes of water	Eight volumes of water
Ash	88·3	79·0	72·0
Nitrogen	91·7	82·9	77·1
Thiamin	93·6	63·3	55·4
Riboflavin	75·0	35·6	25·0
Nicotinic acid	89·5	56·6	50·7
Calcium	91·2	82·4	73·5
Phosphorus	93·4	79·2	76·4
Phytin phosphorus ...	94·0	83·2	78·6
Iron	76·0	55·0	50·0
Available iron	74·0	54·3	52·0

TABLE XVI.

Percentage loss of T.3 (Nagina, U.P.) during washing and cooking.

	In washing	IN GRUEL OF RICE COOKED WITH:	
		Six volumes of water	Eight volumes of water
Ash	5·5	15·1	18·2
Nitrogen	0·8	2·8	4·1
Thiamin	5·5	20·4	30·6
Riboflavin	13·9	23·0	28·4
Nicotinic acid	7·0	31·1	34·4
Calcium	8·5	30·5	36·7
Phosphorus	2·3	11·7	14·0
Phytin phosphorus ...	1·8	8·2	9·7
Iron	21·4	24·0	34·0
Available iron	26·5	21·0	24·0

The cooking time was 40 minutes with just sufficient water (650 c.c.) and with six volumes of water and 46 minutes with eight volumes of water. The average weights of cooked rice were, respectively, 597 g., 585 g. and 587 g. The moisture contents of the corresponding cooked rice samples were 70·5, 72·2 and 72·8 per cent, respectively.

The percentage retention of nutrients in cooked rice (T.3) is shown in Table XVII:—

TABLE XVII.

Percentage retention of nutrients in cooked rice (T·3).

	RICE COOKED WITH:		
	Just sufficient water	Six volumes of water	Eight volumes of water
Ash	94·5	82·4	79·3
Nitrogen	98·1	97·0	96·5
Thiamin	90·9	55·8	46·4
Riboflavin	77·6	63·2	58·5
Nicotinic acid	92·6	61·3	57·8
Calcium	90·5	60·0	54·7
Phosphorus	97·9	87·0	83·3
Phytin phosphorus... ...	95·8	90·0	88·9
Iron	76·0	52·0	48·0
Available iron	72·3	52·3	49·5

TABLE XVIII.

Percentage loss of Charnock (W. Bengal) during washing and cooking.

	In washing	IN GRUEL OF RICE COOKED WITH:	
		Six volumes of water	Eight volumes of water
Ash	10·0	19·0	24·5
Nitrogen	2·2	5·1	6·8
Thiamin	4·9	31·7	41·7
Riboflavin	11·2	22·7	27·0
Nicotinic acid	13·4	20·9	35·1
Calcium	10·5	12·9	19·3
Phosphorus	6·0	13·7	15·2
Phytin phosphorus... ...	3·1	10·4	15·7
Iron	23·5	24·4	30·5
Available iron	25·0	22·0	23·5

The cooking time was 30 minutes in all the cooking experiments with just sufficient (700 c.c.) water, six volumes and eight volumes of water and the weights of cooked rice were 640 g., 545 g., and 575 g., respectively. The mositure content of the corresponding cooked rice samples were 72·8, 71·0 and 72·5 per cent, respectively.

The percentage retention of nutrients in cooked rice is given in Table XIX:—

Table XIX.

Percentage retention of cooked rice (Charnock).

	Rice cooked with:		
	Just sufficient water	Six volumes of water	Eight volumes of water
Ash	89·0	75·5	69·0
Nitrogen	97·4	91·8	89·5
Thiamin	88·2	65·2	51·5
Riboflavin	90·2	66·9	61·8
Nicotinic acid	86·7	65·2	50·8
Calcium	87·9	72·5	69·2
Phosphorus	93·1	80·1	77·0
Phytin phosphorus	96·5	86·0	80·2
Iron	76·7	51·9	44·4
Available iron	74·0	53·5	50·0

3. Nutrition Research Unit under Dr. R. G. Chitre at the Seth G. S. Medical College, Bombay.

During the year under report, work on the nutritive value of pure bred strains of cereals and pulses has been continued. Estimations of nicotinic acid, riboflavin and thiamine by chemical methods and estimation of folic acid by microbiological method have been carried out on the samples obtained from the different centres. Effect of the proteins of certain cereals and pulses on the glycogen, fat and protein in the liver has been studied. Experiments as regards the liberation of amino acids from some of the cereals and pulses have been started. Work on the metabolism of nicotinic acid in human subjects has been continued. Total pyridine nucleotides in blood and excretion of nicotinic acid metabolites in urine have been estimated. Studies in the biosynthesis of nicotinic acid in cereals and pulses have been continued.

I. NUTRITIVE VALUE OF PURE BRED STRAINS OF CEREALS AND PULSES.

(*a*) *Estimations of nicotinic acid, riboflavin and thiamine.*

Eighty strains have already been reported for their nicotinic acid, riboflavin and thiamine contents. This year eighty-three new representative strains of cereals and pulses received from different centres were examined for the contents of the above vitamins. Chemical methods were employed for the estimations. Table I gives the results obtained.

TABLE I.

Thiamine, nicotinic acid, riboflavin content of pure bred strains of cereals and pusles.

Serial number.	Strain number	Thiamine, μg./100 g.	Nicotinic acid, mg./100 g.	Riboflavin, μg./100 g.
1. *Rice*				
(Figures are calculated on the weight of the dehusked grain)				
Pusa				
81	NP-24	260	3·38	130
82	NP-31	220	3·95	130
83	NP-52	272	5·66	259
Cuttack				
84	R-13A	301	3·67	41·5
85	SR-26B	205	2·57	288
86	T-442	183	4·57	210
87	T-141	180	2·34	131
88	B-76	262	2·87	39·6
89	N-136	329	3·96	293
2. *Bajri*				
Coimbatore				
90	PVCO-1	379	3·0	234
Baroda				
91	NO-5	169	1·90	164
3. *Jowar*				
Gwalior				
92	No. 12-2	85·5	1·90	61·0
4. *Ragi*				
Coimbatore				
93	CO-1	106	2·48	144
94	CO-6	158	3·21	223
5. *Varagu*				
Coimbatore				
95	CO-1	93·0	2·86	...
6. *Oats*				
Kanpur				
96	X-27	265	2·98	86·3
97	NP-1	233	1·47	140
98	NPHyb-1	243	2·04	86·3
99	NPHyb-3	217	2·19	80·0
100	NP-2	115	1·73	80·0

TABLE I—(*Contd.*)

Serial number.	Strain number	Thiamine, μg./100 g.	Nicotinic acid, mg./100 g.	Riboflavin, μg./100 g.
		7. *Bengal gram*		
Delhi				
101	T-87	89·3	2·33	64·0
Coimbatore.				
102	CO-1	30·9	2·38	64·0
Kanpur.				
103	NP-17	178	2·11	60·5
104	NP-25	167	2·54	221
105	NP-28	269	3·43	127
106	NP-53	190	3·81	76·0
107	NP-58	185	2·77	252
		8. *Peas*		
Delhi				
108	T-19	410	3·46	414
109	T-16	310	1·73	310
Kanpur				
110	NP-29	210	2·72	446
		9. *Wheat*		
Kanpur				
111	Pb-591	445	3·41	103
112	C-13	287	4·33	163
113	NP-52	383	4·0	91·2
114	NP-125	317	3·39	137
115	NP-710	374	4·0	143
116	NP-715	124	2·59	81·1
117	NP-718	252	1·80	111
118	NP-720	475	4·33	197
119	NP-721	193	2·85	105
120	NP-737	284	2·51	114
121	NP-745	255	2·31	87·3
122	NP-755	51·8	3·14	55·0
123	NP-758	202	3·93	47·6
124	NP-760	674	2·11	107
125	NP-761	296	2·42	91·4
126	NP-762	264	1·83	124
127	NP-764	401	1·90	189
128	NP-768	370	3·41	52·6
129	NP-770	260	4·21	224
130	NP-771	435	1·90	25·3
131	NP-775	445	4·0	123
132	NP-781	168	1·58	93·7
133	NP-782	166	2·85	148
134	NP-798	147	1·06	111
135	NP-799	...	1·58	...

TABLE I—(*Contd.*)

Serial number.	Serial number	Thiamine, μg./100 g.	Nicotinic acid, mg./100 g.	Riboflavin, μg./100 g.
		9. *Wheat*—(concld.)		
Pusa				
136	W-245	404	2·60	195
137	MH-43	318	2·19	253
138	PC-36	164	2·85	292
New Delhi				
139	C-518	330	0·95	99·3
140	C-591	354	0·95	99·3
141	K-13	415	1·65	154
		10. *Chinese mung*		
Kanpur				
142	...	140	3·18	336
		11. *Soya bean*		
Delhi				
143	T-49	130	3·57	320
		12. *Green gram*		
Gwalior				
144	NO-3	85·4	2·11	213
Delhi				
145	T-1	89·8	2·19	176
U.P.				
146	NO-1	150	3·0	132
Coimbatore				
147	CO-1	143	2·93	233
Pusa				
148	NP-18	90·7	4·33	215
		13. *Lentil*		
Kanpur				
149	NPHyb-1	68·5	3·80	169
Pusa				
150	NP-11	134	1·02	160
151	Hybrid	115	0·88	115
		14. *Red gram*		
Gwalior				
152	NO-3	205	1·58	151
153	SA-1	180	1·90	139

TABLE I—(*Concld.*)

Serial number	Strain number	Thiamine, μg./100 g.	Nicotinic acid, μg./100 g.	Riboflavin, μg./100 g.
	Red gram—(concld.)			
Pusa				
154	NP-24	204	4·10	160
155	NP-51	206	5·20	145
156	NP-69	184	3·50	128
157	NP-80	186	3·29	155
	15. *Black gram*			
Gwalior				
158	NO-18	237	2·2	231
Delhi				
159	T-27	37·5	2·6	212
Pusa				
160	NP-4	345	4·30	176
161	NP-6	253	5·80	191
162	NP-7	427	4·50	240
163	NP-14	352	5·10	243

(*a*) *Estimation of folic acid.*

Microbiological assay of folic acid was carried out in the representative strains of the cereals and pulses. Up to now twenty-three strains have been reported for their folic-acid content. This year seventy-four new strains have been examined. The results are embodied in Table II. The method using an enzyme preparation from rat's intestine was employed for these assays (Chitre and Agashe, *Jour. Univ. of Bombay*, **23,** 5, 67, 1955).

TABLE II.

Total folic-acid content of pure bred strains of cereals and pulses.

Serial number	Strain number	Folic acid, μg./g.
	1. *Rice*	
	(Figures are calculated on the weight of the dehusked grain)	
Cuttack		
24	R-13 A	0·335
25	T-812	0·354
26	T-442	0·199
27	T-1242	0·182
28	B-76	0·466
29	N-136	0·286
30	FR-43 B	0·207
31	T-412	0·336
32	CH-47	0·276
33	CH-45	0·276
34	T-608	0·341

TABLE II—(*Contd.*)

Serial number	Strain number	Folic acid, μg./g.
	1 *Rice*—(concld.)	
Aduturai		
35	ADT-2	0·207
36	ADT-3	0·324
37	ADT-20	0·303
Nellore		
38	BCP-1	0·378
Samalkot		
39	SLO-13	0·343
	2. *Bajri*	
Coimbatore		
40	PVCO-1	0·178
41	CO-1	0·155
42	CO-3	0·162
43	CO-4	0·152
Baroda		
44	NO-5	0·158
	3. *Jowar*	
Gwalior		
45	NO-122	0·061
Coimbatore		
46	CO-1	0·058
	4. *Tenai*	
Coimbatore		
47	CO-1	0·066
48	CO-3	0·062
	5. *Ragi*	
Coimbatore		
49	CO-1	0·053
50	CO-6	0·050
	6. *Varagu*	
Coimbatore		
51	CO-1	0·064
	7. *Oats*	
Kanpur		
52	X-27	0·303
53	NP-1	0·325
54	NPHyb-1	0·327
55	NPHyb-3	0·280
56	NP-2	0·310

TABLE II—(*Contd.*)

Serial number	Strain number	Folic acid, µg./g.
	8. *Bengal gram*	
Delhi		
57	T-87	0·178
Coimbatore		
58	CO-1	0·162
Kanpur		
59	NP-17	0·178
60	NP-25	0·182
61	NP-28	0·172
62	NP-53	0·168
63	NP-58	0·151
	9. *Peas*	
Delhi		
64	T-19	0·150
65	T-16	0·075
Kanpur		
66	NP-29	0·075
	10. *Wheat*	
Kanpur		
67	PB-591	0·282
68	NP-52	0·312
69	NP-710	0·316
70	NP-715	0·304
71	NP-718	0·292
72	NP-720	0·321
73	NP-721	0·322
74	NP-737	0·305
75	NP-745	0·390
76	NP-755	0·287
77	NP-758	0·312
78	NP-760	0·321
79	NP-761	0·321
80	NP-762	0·298
81	NP-764	0·302
82	NP-768	0·312
83	NP-770	0·313
84	NP-771	0·298
85	NP-775	0·287
86	NP-781	0·306
87	NP-782	0·313
88	NP-798	0·306
89	NP-799	0·321

TABLE II—(*Concld.*)

Serial number	Strain number	Folic acid μg./g.
	11. *Soya bean*	
Delhi 90	T-49	0·499
	12. *Green gram*	
Gwalior 91	NO-3	0·124
Delhi 92	T-1	0·120
U.P. 93	NO-1	0·116
Coimbatore 94	CO-1	0·180
	13. *Red gram*	
Gwalior 95	NO-3	0·240
	14. *Lentil*	
Kanpur 96	NPHyb-1	0·080
	15. *Black gram*	
Gwalior 97	NO-18	0·295

(*c*) *Effect of proteins of certain cereals and pulses on the glycogen, fat and protein content of liver in albino rats.*

Some pure strains of cereals and pulses were fed as sole sources of protein to young albino rats at ten per cent level and the effect on the liver glycogen, fat and protein was noted after an experimental period of ten weeks. The results are given in Table III.

It is observed that in general, proteins giving a better growth formed greater amounts of liver protein as compared to those giving lesser growth. A ratio 'R' was calculated showing the relation between the average hepatic protein, glycogen and fat and the growth of the animal. This was found to correspond to the order of the growth observed. In Table III, biological values of foodstuffs are also given for comparison. It is observed that the ratio 'R' gives a better index of the utilization of the protein for growth than the biological value. The ratio obtained for casein has been equated to hundred and values for the other foodstuffs calculated accordingly.

TABLE III.

Protein source	‡R	Increase in body-weight in g.	Biological value	LIVER		
				Protein	Fat	Glycogen
				(results are expressed in g. per cent)		
Casein	100·0	85·3	100·0	22·86±0·53	3·46±0·41	2·65±0·29
Wheat ...	64·7	71·2	66·0	21·77±0·76	4·32±0·86	3·18±0·51
Bengal gram	35·2	62·0	76·0	18·41±0·67	3·93±0·55	6·21±0·55
Rice ...	28·0	40·7	80·0	19·60±0·81	5·90±1·43	3·0 ±0·96
Green gram	16·6	30·5	51·0	18·83±0·55	4·29±0·21	5·84±0·55
Bajri ...	14·0	27·3	83·0	17·62±1·22	5·36±1·79	5·43±0·44
Lentil ...	9·0	24·0	58·0	16·61±0·42	5·38±0·29	5·54±0·34
Jowar ...	6·4	15·1	83·0	15·65±1·54	6·35±2·02	5·08±0·44
Ragi ...	4·6	14·8	89·0	12·40±0·39	6·78±0·56	8·27±0·40
Red gram	6·5	10·7	74·0	17·14±0·45	4·75±0·25	5·16±0·07
Tenai ...	1·0	2·5	77·0	*13·87	*5·09	*5·14
Horse gram	†	—5·0	*59·0	*14·41	*8·76	*5·01

* Not statistically treated as the number of surviving animals was too small.

† In this case the ratio proved to be negative as the growth was also negative.

‡ $R = \frac{\text{Protein}}{\text{glycogen} + \text{fat}} \times \text{growth}.$

(*d*) *Enzymic liberation of amino acids from certain foodstuffs.*

As indicated in the last year's report, physiological availability of adequate amounts of amino acids of certain foodstuffs might be playing an important role in the formation of tissue proteins. Experiments have been undertaken to study the sequence of release of different amino acids by enzymic digestion *in vitro* from the foodstuffs. Four foodstuffs—rice, bajri, Bengal gram and red gram have been examined for the sequence of release of amino acids. The results are being scrutinized.

II. STUDIES IN NICOTINIC ACID METABOLISM.

(A) NICOTINIC ACID METABOLITES IN HUMAN SUBJECTS.

(*a*) *Total pyridine nucleotides in blood.*

Work that was taken in hand last year was continued. It was found that the technique for the D.P.N. and T.P.N. estimations is much more time-consuming and requires special and not easily available reagents and materials in comparison to the technique required for the estimation of total pyridine nucleotides. It was thought, therefore, worthwhile to see whether the estimation of total pyridine nucleotides in the blood would serve the purpose of detecting the faulty metabolism of the vitamin in diseased conditions.

Forty bloods of normal healthy Indian male subjects have been examined for their total pyridine nucleotides. The subjects were Blood

Bank donors, their ages being between twenty and thirty years. Hæmoglobin, hematocrit and blood group determinations were carried out. The total pyridine nucleotides were estimated by the method of Huff *et al.* (*Jour. Biol. Chem.*, **167,** p. 169, 1947). The Table IV gives the average results obtained.

TABLE IV.

Number of cases	Hb. g. per cent	Hematocrit c.c. per cent	TOTAL PYRIDINE NUCLEOTIDES:	
			γ/c.c. of blood	γ/c.c. of erythrocytes
40	13·96	43·50	23·69	48·26

(*b*) *Capacity of the red blood cells to synthetize the pyridine nucleotides.*

In addition to the above estimations of the total pyridine nucleotides, experiments have been undertaken to assess the capacity of the red blood cells to synthetize the pyridine mononucleotides by addition of nicotinamide. This will be studied in both health and diseased conditions. It is hoped that this capacity would differ in health and disease and would be indicative of the latter.

(*c*) *Excretion of nicotinic acid metabolites in human subjects.*

The following metabolites were estimated in the urine: (1) Nicotinic acid, (2) nicotinamide, (3) nicotinuric acid, (4) N-1-methylnicotinamide, (5) 6-pyridone of N-1-methylnicotinamide and (6) trigonelline.

Urines of fifty-eight normal healthy young women of ages between eighteen and thirty years have been examined. The results are given in Table V. Estimation of the metabolites in the urines of pregnant women also has been undertaken. 6-pyridone of N-1-methylnicotinamide figures could not be calculated due to the absence of the standard.

TABLE V.

Average total urinary excretions in mg. during twenty-four hours.

Number of subjects	N-1-methylnicotinamide	Nicotinic acid	Nicotinamide	Nicotinuric acid	Trigonelline
58	3·70	0·54	0·92	0·61	0·035

(*d*) *Total nicotinic acid in the blood of normal subjects.*

During the year under report total nicotinic-acid content of twenty-four normal Indian subjects has been estimated. For these estimations, the chemical method (Abdel Kader and Ellinger, *Biochem. Jour.*, **44,** p. 77, 1949; modification by Chitre and Desai, 'Symposium on Vitamins', Bangalore, 1955), has been used. The average results are given in Table VI. Comparative values for four of these subjects were obtained by the use of a microbiological method also. They agreed fairly well with each other.

TABLE VI.

Total nicotinic acid in blood of normal Indians.

Number of subjects	Age in years	Total nicotinic acid, mg./100 c.c.	Range, mg. per cent
24	20 to 30	0·56	0·33 to 0·78

(B) BIOSYNTHESIS OF NICOTINIC ACID IN GERMINATING CEREALS AND PULSES.

In the last year's report a chain of biosynthesis was indicated tentatively. Experiments with indole were carried out, but no rise in nicotinic acid was observed. During the year, values for nicotinic acid metabolites by chemical method reported earlier were verified by microbiological assays.

It is intended to carry out experiments to ascertain the exact sequence of reactions by the use of radio-active tryptophane. Request has been already forwarded to the Atomic Energy Commission through the Director, Indian Cancer Research Centre, Bombay, for a grant to obtain the radio-active tryptophane. The decision of the Atomic Energy Commission is awaited.

4. Inquiry on the metabolism of vitamin A under Dr. K. Rajagopal at the All-India Institute of Hygiene & Public Health, Calcutta.

While there is still no definite information concerning the exact biochemical rôle of vitamin A, there are numerous observations, disclosing its major importance to epithelial structures, retina and the processes of general growth and maintenance. It is also associated with the function of some hormones, e.g., thyroxine. Even though it is not known how vitamin A functions within the cell, its presence in the water soluble form in the mitochondria and other cytoplasmic granules has been demonstrated.

Albumin-retinene complex.—The most difficult task associated with investigation on metabolism of vitamin A is that, being a fat soluble substance, vitamin A cannot be directly used in tissue metabolism studies. Hence, the first requisite is to get vitamin A in a water soluble state, quite homogeneous to the body fluids. This was partly achieved in this Institute by combination of vitamin A aldehyde with plasma albumin. The biological activity of this compound albumin-vitamin A-aldehyde complex has been thoroughly studied here and reported previously. From the results obtained it is evident that this water soluble complex is better utilized than vitamin A-acetate (Datta and Rajagopal, *Alumini Assoc. Bull.*, A.I.I.H. & Pub. Hlth., July, 1954, p. 11.

In order to investigate the nature of combination between the albumin and vitamin A-aldehyde, the aqueous solution of the complex was studied spectrophotometrically.

The absorption spectra indicated the presence of the two characteristic peaks, one for albumin and the other for vitamin A-aldehyde (slightly shifted). This shows that the complex formed is in a true solution. Absence of tyndall-effect in the visible region of the spectra also indicates that this is not a colloidal solution. Here vitamin A-aldehyde, which is a fat-soluble substance has passed into the water soluble state in combination with a protein. The conditions of preparation, the nature of the reactive groups and the water solubility suggest that the two components are chemically combined. But there is no confirmatory evidence.

The only way to solve this problem is to study the electrokinetic behaviour. If the two complex migrate in an electric potential, as a single unit, then it can be taken for granted that the two components are chemically combined.

The standard equipment for this type of investigation is Tiselius electrophoretic apparatus. Due to non-availability of such an equipment, attempts have been made here to utilize the recently developed technique of micro-electrophoresis on filter paper to solve this problem.

The apparatus assembled here is according to the principle of Albanese *et al.* (*Arch. Biochem. & Biophysics*, **54,** p. 304, 1955) with certain modifications. This was constructed from some glass plates (6 mm. thick) and two staining troughs. The bottoms of the staining troughs, which serve as electrode vessels were covered with paraffin-wax and three vertical glass baffles were placed to stop free diffusion, so that no electrode products can reach the ends of paper during the course of separation.

The electrodes used are platinum foils, fused into glass-tubes. The whole system is covered with glass cover to prevent free evaporation from paper strip.

The current is supplied from a selenium rectifier power supply unit with provision for variable d.c. voltage (50 to 180 v.). A millimeter is connected in series to record the current passed. Strips are cut from large sheets of Whatman No. 1 filter paper to measure 4 × 50 cm. The strips are marked by drawing a pencil line, 12 cm. from the negative end.

The albumin-retinene complex solution is placed on this line at two points in equal amounts at a distance of 1 cm. from each side. After the samples have air-dried slightly, the strip is stretched across a bridge. The paper is then moistened with veronal buffer (pH 8·6) with a brush. The bridge is now placed on the electrode vessel, containing veronal buffer, so that the positive and negative ends of the paper strips dip in the respective electrode chambers. The electrodes are connected to the power supply unit and the voltage is regulated to allow a current of 2·0 mA to 3·0 mA. The glass cover is then placed over the apparatus and the current is allowed to pass for four to five hours.

After this the paper strip is withdrawn carefully and allowed to dry up in a vacuum desiccator. The paper is then cut length-wise into two long strips at the centre, each containing a single but identical run, one for the alubmin and the other for vitamin A-aldehyde, each of which can be spotted only by different reagents. For albumin, one half is heated to 120° for ten minutes and then stained with bromophenol-blue-zinc-sulphate solution, washed and dried. The portion occupied by albumin will only be coloured. The centre of this zone is marked. The other strip is sprayed with $SbCl_3$ solution when the zone occupied by vitamin A-aldehyde is coloured blue (but this colour is transient) and this zone is marked with a pencil.

The distances of the centres of the protein zone and the $SbCl_3$ coloured zone from the original starting points are measured carefully.

The distances in pair experiments were found to be almost equal.

Experiment I	Experiment II	Experiment III
3·52 cm.	4·05 cm.	4·35 cm.
3·50 cm.	4·17 cm.	4·52 cm.

If vitamin A-aldehyde be not in chemical combination with the protein moiety, then in the electrical field it should not have migrated along with the protein. From the experiment, it is found that they have traversed equal path and this can only be explained if albumin is in chemical combination with vitamin A-aldehyde.

Vitamin-A like activity of poly-unsaturated fatty acids.—It was observed in this laboratory that linseed oil and linoleic acid have got some beneficial effect on follicular hyperkeratosis (phrynoderma) and can prolong the life of vitamin A-deficient rats. This problem has been further investigated this year. It is found that when linoleic acid is purified from isomers and any foreign substance by chromatography, it has little or no protective action on vitamin A-deficient rats. Technical quality of this linseed oil and linoleic acid were examined spectroscopically and found to contain some fractions with characteristic absorption. In order to identify and concentrate these fractions, a test experiment was performed in which linseed oil was distilled at a vacuum of 1×10^{-4} mm. (Hg.). Two fractions were distilled over and tested biologically. Both the fractions were, however, found to have no biological activity similar to vitamin A. By spectroscopic examination, it was found that the fraction showed non-specific absorption in the region 253 mμ to 270 mμ.

Another sample of linseed oil was isomerized to make the double bonds conjugated. It was also tested biologically on vitamin-A deficient rats. It was found that this isomerized linseed oil has got some beneficial effect on vitamin-A deficient rats.

5. Inquiry on the metabolism of nicotinic acid under Dr. S. Banerjee at the Presidency College, Calcutta.

Further observations on the biosynthesis of nicotinic acid from tryptophane in guinea-pigs and rabbits.—The metabolism of nicotinic acid and tryptophane is not identical in mammals. In previous reports it was shown that trigonelline, a derivative of nicotinic acid which, however, is biologically inactive, is not a metabolic end product of nicotinic acid in rats, rabbits, guinea-pigs and monkeys. Rabbits fed trigonelline excreted only 50 per cent of the substance in urine. The fate of the rest could not be accounted for. It was also reported that liver was the principal site of synthesis of nicotinic acid from tryptophane in rats and guinea-pigs were inefficient converters of tryptophane to nicotinic acid. In continuation of the above work the metabolism of tryptophane and trigonelline was studied in guinea-pigs after their liver was injured by injections of carbon tetrachloride. The metabolism of tryptophane was also studied in rabbits. The results are given in Tables I to III:—

TABLE I.

Twenty-four hours' urinary excretion of nicotinic acid (NA), quinolinic acid (QA), nicotinuric acid (NUA) and trigonelline (TN) by a guinea-pig fed tryptophane or TN before and after injection of carbon tetrachloride (0·03 *c.c./kg. body-weight*).

(Average of three days' excretion by a group of eight animals.)

Supplement	NA, γ	QA, γ	NU, γ	TN, per cent
Nil	43	128	31	24
dl-tryptophane (1 per cent level in diet) ...	60	193	28	24
dl-tryptophane with injection of CCl_4 ...	35	262	54	22
Trigonelline (daily dose of 15 mg. for two days) ...	47	152	17	20
Trigonelline with injection of CCl_4	51	148	14	28

It will be seen from data in Table I that injection of CCl_4 in guinea-pigs after feeding tryptophane slightly increased the excretion of quinolinic acid and nicotniuric acid and the excretion of nicotinic acid was diminished. In rats, however, the injection of CCl_4 inhibited the formation of both nicotinic acid and quinolinic acid. Administration of trigonelline, before and after injections of CCl_4 did not affect the urinary excretion of nicotinic acid for quinolinic acid.

TABLE II.

Twenty-four hours' urinary excretion of trigonelline after feeding trigonelline (15 mg. per day for two days) before and after injections of CCl_4 *in a dose,* 0·03 *c.c. per kg. body-weight.*

Days	TRIGONELLINE IN MG.:	
	Before injection	After injection
1 ...	4·09	4·42
2 ...	4·56	5·50
3 ...	2·15	2·60
4 ...	1·20	1·49

Table III.

Twenty-four hours' urinary excretion of nicotinic acid (NA), quinolinic acid (QA), N-methyl nicotinamide (N-MN) and trigonelline (TN) by a rabbit before and after feeding tryptophane.

(Average excretion of three rabbits.)

Supplement	NA, γ	NA, γ	N-MN, γ	TN, mg.
None	212	626	122	12
dl-tryptophane-250 mg. for two days	363	3,712	140	12

Table II shows that after injections of CCl_4 administration of triognelline produced an increased excretion of trigonelline only on the 2nd day of the injection but the sum total of trigonelline excreted for four days was more or less similar. Guinea-pigs excreted only 25 per cent of ingested trigonelline.

Table III shows that rabbits are better converters of nicotinic acid from tryptophane than guinea-pigs.

Absorption spectra of the urine of guinea-pigs after feeding tryptophane.—Absorption spectra of urine of guinea-pigs after feeding tryptophane were studied in order to know the intermediate product of tryptophane metabolism. The nature of the curve was similar to that given by 3-hydroxy kynurenine. The absorption maximum was at 380 mμ and the same for pure 3-hydroxy kynurenine is 370 mμ. The shifting of the curve might be due to some impurities present.

Metabolism of nicotinic acid and nicotinamide when fed at a higher level in the diet.—When nicotinamide was fed at a high level, the growth of rats was inhibited and the animals died. Nicotinic-acid metabolites excreted in the urine of these animals were not studied. The deleterious effect of nicotinamide was explained differently by different workers. The effect of feeding nicotinic acid and nicotinamide on the urinary excretion of nicotinic-acid derivatives was studied. The effect of addition of methionine, folic acid and tryptophane with the basal diet on the urinary excretion of nicotinic-acid derivatives after the feeding of nicotinamide was also studied. The results are given in Table IV.

After feeding 100 mg. nicotinic acid daily for four days the growth of 12 rats studied was inhibited, but there was no fall in the weight of the animals. When the same dose of nicotinamide was fed all the animals lost weight. The excretion of nicotinic acid was not marked after feeding nicotinamide. The excretion of N-MN and quinolinic acid was higher in rats fed nicotinamide than in rats fed nicotinic acid. The increased excretion of N-U might indicate increased loss of methyl groups from the body. But when the diet was supplemented with methionine which served as a methyl donor and with folic acid, which helped in the synthesis of methyl groups, the administration of nicotinamide produced a fall in the weight of rats to the same degree. These observations indicated that growth inhibition was not due to loss of methyl groups from the body. It is difficult to explain the increased urinary excretion of quinolinic acid after feeding nicotinamide. Further, study is in progress to explain this.

TABLE IV.

Twenty-four hours' urinary excretion of nicotinic acid (NA), quinolinic acid (QA), nicotinuric acid (NU), N-methyl nicotinamide (N-MN) by a rat fed 100 *mg. NA or NA-amide.**

Supplement	NA, mg.	QA, mg.	NU, mg.	N-MN, mg.
100 mg. NA daily for four days	73·5	24·3	4·4	0·71
100 mg. NA-amide for four days	15·3	40·6	7·9	4·37
Methionine at two per cent level with 100 mg. NA-amide	14·2	33·9	6·2	6·75
Methionine at two per cent level and folic acid 0·4 per cent level with 100 mg. NA-amide	19·8	38·2	5·1	4·76
Tryptophane at one per cent level with 100 mg. NA-amide	19·1	33·1	5·9	5·28

*Average excretion per day by 10 rats.

Urinary excretion of ketone bodies and creatinine in rats after feeding high dose of nicotinic acid or nicotinamide.—The urinary excretion of ketone bodies and creatinine in rats after they were fed nicotinic acid or nicotinamide were also determined in order to know if the deleterious effect of feeding high dose of nicotinamide was due to the rise of ketone bodies or creatinine in the blood. The results are shown in Table V.

TABLE V.

Twenty-four hours' urinary excretion of creatinine and ketone bodies by a rat after administration of nicotinic acid or amide.

(Average excretion of ten rats.)

Supplement	Creatinine, mg.	Ketone bodies, γ
None	1·56	66
100 mg. NA (four days)	9·30	518
100 mg. NA-amide (four days) ...	2·58	134
100 mg. NA-amide with methionine (two per cent level)	3·00	110
100 mg. NA-amide with tryptophane (one per cent level)	3·37	154

Feeding nicotinic acid increased the urinary excretion of both creatinine and quinolinic acid but after nicotinamide the excretion of these substances was not marked. The deleterious effect of nicotinamide, therefore, was not due to the formation of these substances in increased amounts in the body.

6. Inquiry on human requirements of riboflavin and thiamine under Dr. H. N. De at the M. G. M. Medical College, Indore.

1. STUDIES ON RIBOFLAVIN METABOLISM WITH TYPICAL INDIAN DIETARIES IN NORMAL FEMALE SUBJECTS.

A series of experiments to explore the minimum requirements of riboflavin for normal Indian females and also to assess the adequacy of the typical dietaries to fulfil this requirement has been carried out with a different technique by the study of urinary return of test dose given by intramuscular injection. A part of the work of this series was reported last year and in this year ; further work was continued and results are presented in Table I. Twelve normal female nurses were selected for this work. They were kept on diet, based on rice, whole wheat, milk, pulses and vegetables, supplying about 1·33 mg. of riboflavin per day. These subjects were divided in two batches of six each and experiment was conducted on each batch separately. In each batch two test-dose expertiments were performed after a lapse of two months' period to find out as to how far the above level of dietary riboflavin can satisfy the riboflavin requirement. If the per cent test-dose return in urine in each subject after a lapse of two months feeding on this diet remained almost in the same level, then the above dietary regime should be regarded as adequate to meet the minimum requirements. Dietary supply of riboflavin below the minimum level will tend to show a decrease in percentage return of test dose after two months feeding on such subminimal riboflavin diet.

Results presented in Table I show that on an intake of 1·33 mg. of riboflavin at an initial period the nurses excreted in urine on an average of 0·118 μg./c.c. as basal excretion. The per cent return of 2 mg. test dose at this period showed an average value of 6·35. When similar experiment was performed on the same nurses after two months' continuation on the same diet, the basal excretion showed an average value of 0·137/μg./c.c. and the test dose given showed a return of 6·01 per cent. From these values it is revealed that the subjects were excreting the same amount of riboflavin in per c.c. of basal excretion and the per cent test dose return also remained the same even when the diet was continued for a period of two months. This shows that the above dietary level of 1·33 mg. as supplied by typical vegetarian rice and whole wheat diet is adequate to satisfy the minimum requirements. This also substantiates our previous observations reported in the previous year.

Another group of nurses, at present, have been kept on a diet consisting of rice, pulses, milk and vegetables supplying about 0·9 mg. of riboflavin to see how far this level can satisfy the minimum requirements and the results will be submitted in due course.

TABLE I.

Showing the riboflavin excretion and test dose return on moderate intake of riboflavin in female nurses at different periods. Test dose given—2 mg. by intramuscular injections.

Serial number and name of subject	Dietary intake of riboflavin, mg./day	BEFORE TEST DOSE:			AFTER TEST DOSE:			Percentage excretion of test dose
		Volume of urine, c.c.	Riboflavin excretion		Volume of urine, c.c.	Riboflavin excretion		
			μg/c.c.	Total/μg.		μg/c.c.	Total/μg.	
	PERIOD I.							
1. Agenes	1·33	50	0·075	3·8	42	2·7	113·4	2·28
2. Chandra	1·33	120	0·069	8·28	115	1·94	223·5	10·25
3. Emelin	1·33	105	0·130	13·75	60	2·30	138·0	6·5
4. Hilda	1·33	45	0·087	3·94	130	2·00	280·0	12·4
5. Krishna	1·33	105	0·169	17·53	115	1·08	124·2	5·2
6. Leela	1·33	110	0·0285	3·13	60	1·475	88·5	4·34
7. Maya	1·33	50	0·097	4·88	325	0·55	178·7	7·5
8. Nalini	1·33	124	0·096	11·90	120	1·42	170·4	7·98
9. Sarojini	1·33	60	0·193	11·58	75	1·30	97·5	4·1
10. Susan	1·33	115	0·080	9·20	108	1·36	146·8	7·25
11. Sumati	1·33	113	0·060	7·00	45	1·88	84·6	4·09
Average			0·118					6·85
	PERIOD II (After two months).							
1. Agnes	1·33	380	0·092	34·96	190	0·712	135·3	5·9
2. Chandra	1·33	93	0·130	9·49	133	0·902	101·0	5·1
3. Emelin	1·33	105	0·086	9·03	245	1·15	289·0	13·0
4. Hilda	1·33	390	0·102	39·78	100	3·072	307·2	15·0
5. Krishna	1·33	125	0·155	19·375	...	1·166	128·3	5·56
6. Leela	1·33	...	...	...	...	...	...	...
7. Maya	1·33	190	0·184	34·920	90	1·523	137·1	6·03
8. Nalini	1·33	68	0·137	9·32	53	1·560	82·6	3·7
9. Sarojini	1·33	305	0·068	20·74	65	4·60	299·0	12·7
10. Susan	1·33	225	0·260	58·50	150	0·893	121·9	4·75
11. Sumati	...	...	...	...	...	...	...	...
Average			0·137					6·01

2. RIBOFLAVIN METABOLISM BY NORMAL FEMALE SUBJECTS ON LOW RIBOFLAVIN DIET.

It has been reported last year that the two male subjects subsisting on a low riboflavin diet—supplying 0·768 mg. of riboflavin per day, could not maintain the saturation level after the saturation and experimental doses were withdrawn and the subjects were kept only on the basal diet for a prolonged period. This apparently indicated that this level of riboflavin was not probably adequate to supply the minimum requirement of riboflavin for normal Indian male subjects although dietary level between 0·83 and 1·2 mg. as supplied by typical Indian vegetarian and non-vegetarian diet could satisfy the requirement.

In the year under review further work in this line has been continued with four female subjects kept on a diet of low riboflavin contents of 0·71 mg. per day based on highly milled rice, pulses and vegetables. In carrying out the experiment on female subjects the menstrual period was taken in account as the riboflavin assay of urine samples during this period might give incorrect values. Whenever the menstrual period just superimposed upon the scheduled urinary collection period, the collection was kept suspended until completion of menstruation and during this period the saturation and other doses, if started before, were also kept continued. Results of this series of experiments are presented in Table II, from which it will be noted that in basal diet period (period I) they excreted an average of 13·0 per cent of dietary riboflavin in urine. Test dose return on basal diet in period II, showed also a low level of 4·3 per cent.

While surveying the per cent test dose return after saturation period (period III), it has been further noted that none of the subjects attained the saturation level as evident from the low test dose return of 21·1 per cent on average in this period. After saturation dose in period IV, only one subject—Sarla, attained the saturation state as evident from the higher return of test dose above 50 per cent. Another subject, Leela, just approached the border level of saturation state. This subject excreted 40·1 per cent of the test dose. The subjects, Nalini and Sumati, could not attain the saturation state even after the second week of saturation as evident from the low return of test dose to the extent of 21·3 per cent in case of Nalini and 27·7 in case of Sumati.

When the saturation dose was withdrawn as in case of experimental dose periods V and VI and last basal diet period VIII, subjects could not maintain even up to the level of saturation which they attained in second week of saturation (i.e. period IV) and gradually the per cent test dose return dropped until it reached a value of 8·3 per cent on an average in the last basal diet period VIII. This inability of the subjects to attain and maintain the saturation state even in the last basal diet period under dietary intake vis-a-vis the ability of the subject to maintain the 50 per cent saturation state on diets supplying 0·83 mg. to 1·36 mg. per day of riboflavin as reported in our previous investigation, clearly indicate that the present supply of 0·71 mg. per day was not sufficient to meet with the tissue saturation requirement for the above female subjects.

Thus, reviewing these data and comparing these against those presented before on intakes of 0·76 mg. to 0·83 to 1·36 mg. of riboflavin, it may be tentatively assumed that the minimum requirement of this vitamin for normal male and female adults may not be less than 0·8 mg. per day.

TABLE II.

Showing the riboflavin metabolism on low riboflavin diet (0·71 mg. per day) in normal female adults.
(The values for excretion represent the daily average expressed in μg.)

Serial number and name of subject	Nature of the diet	Dietary intake, mg.	BASAL EXCRETION PERIOD I		BASAL EXCRETION ON TEST DOSE PERIOD II		SATURATION PERIOD WITH 10 MG.				EXPERIMENTAL PERIODS:					
							Period III		Period IV		Period V 2 mg. daily dose		Period VI 1 mg. daily dose		Period VII basal nil dose	
			Total	As per cent intake	Total excretion 5 mg. test dose	Per cent test dose	Total excretion 5 mg. test dose	Per cent of test dose	Total excretion 5 mg. test dose	Per cent of test dose	Total excretion 5 mg. test dose	Per cent of test dose	Total excretion 5 mg. test dose	As per cent of test dose	Total excretion 5 mg. test dose	As per cent test dose
1. Leela	Vegetarian rice and whole wheat diet.	0·71	117	16·5	Collection not proper.		760	12·8	2,164·5	40·1	1,426	26·2	1,122	20·1	272	3·1
2. Nalini		0·71	82	11·5	209	3·7	1,160	19·0	1,274	21·3	1,262	21·6	720	10·2	809	12·0
3. Sarla		0·71	73·5	10·3	273·5	4·1	1,630·8	31·1	2,759	53·7	837	15·3	1,062	19·7	550	9·5
4. Sumati		0·71	97·5	13·7	356·5	5·2	1,186	21·6	1,483·5	27·7	522	8·5	634	10·7	560	9·2
Average	...	0·71		13·0	...	4·3	...	21·1	...	35·7	...	17·7	...	15·2	...	8·3

3. MINIMUM RIBOFLAVIN REQUIREMENTS OF YOUNG GIRLS (TABLE III).

In last year a series of experiments were started to assess the minimum requirements of young girls. The technique was based on keeping the subjects on a very low riboflavin content diet and gradually increasing the dietary level with simultaneous measurement of the per cent urinary return of test dose given by intramuscular injection at each level of riboflavin intake. The level of intake at which the per cent return of test dose would stabilize will be considered as the minimum requirement value. In the last year's report (as was presented in Table V), it was shown that on an intake of 0·7 mg. of riboflavin per day the subjects excreted on an average 0·139 μg./c.c. in basal excretion and the per cent test dose return in this group of girls amounted to 3·03 on an average. Further continuation of the experiment on the same diet did not produce any appreciable change in per c.c. basal excretion as well as in the per cent test dose return.

Supplementation of this diet with 0·1 mg. of additional riboflavin per day thus bringing the total dietary intake to 0·8 mg. per day and keeping the subjects on this diet for two weeks effected an increase in per c.c. basal excretion and per cent test dose return to the limit of 0·226 mg. per c.c. and 14·57 per cent, respectively.

In the year under review, this work was further continued by increasing the total intake to 0·9 mg. and 1·0 mg. per day. Supplementation of the basal diet with 0·2 mg. of riboflavin thus bringing the total intake to 0·9 mg. per day kept the average values of per c.c. basal excretion and per cent test dose return within the limit of 0·187/μg./c.c. and 12·9 per cent, respectively. Further increase of supplementary dose to 0·3 mg. per day thus bringing the total intake to 1·0 mg. per day did not produce any appreciable change in the per c.c. basal excretion and per cent test dose return. After surveying the above results, it is observed that both the per c.c. basal excretion and per cent test dose return tend to stabilize at a total intake of 0·8 to 0·9 mg. per day. The relationship between the intake level and the per cent test dose return is shown in the Figure and it thus appears that the minimum daily requirement of riboflavin for young girls may lie between 0·8 and 0·9 mg.

INFORMATIONS GATHERED ON RIBOFLAVIN METABOLISM STUDIES.

The following are the main informations gathered in the investigations on riboflavin metabolism with typical Indian dietaries during the period from July, 1952 to September, 1955.

(*i*) The typical Indian vegetarian and non-vegetarian dietaries based on cereals, such as rice, wheat and jowar supply on an average 0·83 to 1·33 mg. of riboflavin per day.

(*ii*) By using an improved method based on saturation oral-test dose-return technique it has been observed that the above level of dietary is sufficient to meet the minimum requirement of this vitamin for normal male Indian adults (De, Datta and Mankad, *Ind. Jour. Med. Res.*, **43,** 3, July, 1955).

(*iii*) By further experiments with adult males and females with low riboflavin diet containing 0·712 to 0·76 mg. of riboflavin, it has been observed that this level is not sufficient to meet the

TABLE III.

Showing riboflavin excretion and test dose return on low intake in orphan girls at different periods. Test dose given 2 mg. by intramuscular injection.

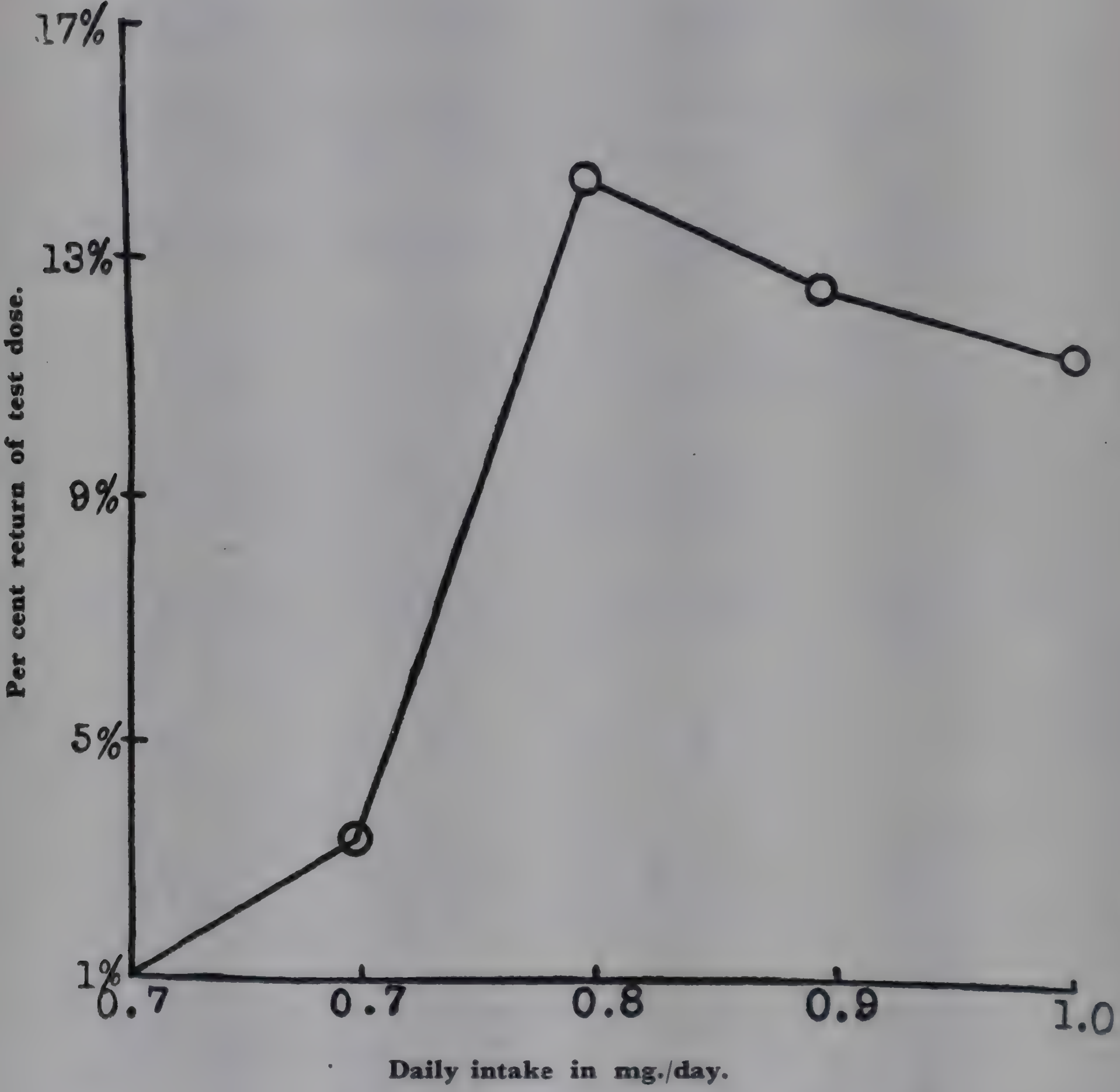
Serial number and name of the subject	Date of the experiment	Dietary riboflavin intake, mg./day	Period	BEFORE TEST DOSE (BASAL EXCRETION):			AFTER TEST DOSE:			Per cent excretion of test dose
				Volume of urine, c.c.	Riboflavin excretion		Volume of urine, c.c.	Riboflavin excretion		
					μg./c.c.	Total μg.		μg./c.c.	Total μg.	
1. Koshallya ...	July, 1954	0·7	I	200	0·23	46·0	95	1·22	115·9	4·75
2. Dhanna	,, ,,	0·7		430	0·04	17·2	55	0·82	45·1	2·14
3. Devki	,, ,,	0·7		92	0·11	10·1	225	0·20	45·0	1·00
4. Lakshmi	,, ,,	0·7		115	0·076	8·7	125	0·49	61·2	2·58
5. Kamla	,, ,,	0·7		130	0·23	25·9	325	0·57	185·2	5·53
6. Kunda	,, ,,	0·7		122	0·21	25·6	175	0·46	80·5	2·19
Average ...					0·149					3·03
1. Koshallya ...	Sept., 1954	0·7	II	44	0·15	6·6	90	1·30	117·0	5·10
2. Devki	,, ,,	0·7		42	0·50	21·0	110	0·96	105·6	2·50
3. Lakshmi	,, ,,	0·7		95	0·097	9·2	180	0·33	59·4	2·10
4. Kamla	,, ,,	0·7		150	0·190	21·8	250	0·53	135·0	4·4
5. Kunda	,, ,,	0·7		75	0·053	3·9	44	0·97	42·6	2·0
Average ...					0·168					3·2

TABLE III (*Concld.*).

Serial number and name of the subject	Date of the experiment	Dietary riboflavin intake, mg./day	Period	BEFORE TEST DOSE (BASAL EXCRETION):			AFTER TEST DOSE:			Per cent excretion of test dose
				Volume of urine, c.c.	Riboflavin excretion		Volume of urine, c.c.	Riboflavin excretion		
					μg/c.c.	Total μg.		μg./c.c.	Total μg.	
1. Koshallya ...	Oct., 1954	0·7 *plus* 0·1 =8 mg.	III	90	0·32	28·8	144	2·36	330·4	14·4
2. Devki	,, ,,			95	0·20	19·0	190	1·73	328·7	14·5
3. Lakshmi	,, ,,			85	0·048	4·1	225	1·66	373·5	18·1
4. Kamla	,, ,,			100	0·11	11·0	210	1·19	249·0	11·3
5. Kunda	,, ,,			190	0·45	40·5	...	...	...	...
Average ...					0·226					14·57
1. Koshallya ...	Nov., 1954	0·7 *plus* 0·2 =9 mg.	IV	150	0·36	54·0	110	3·21	353·1	15·7
2. Devki	,, ,,			380	0·198	75·2	100	2·22	222·0	10·1
3. Lakshmi	,, ,,			290	0·36	26·4	370	0·94	346·8	15·7
4. Kamla	,, ,,			190	0·260	30·4	190	1·28	248·2	9·7
5. Kunda	,, ,,			210	0·024	4·64	370	0·77	284·9	13·6
Average ...					0·187					12·95
1. Khoshallya ...	Dec., 1954	0·7 *plus* 0·3 =1·0 mg.	V	50	0·402	20·1	100	2·18	218·4	8·9
2. Devki	,, ,,			100	0·302	30·2	50	3·89	233·4	10·0
3. Lakshmi	,, ,,			292	0·098	28·6	200	1·45	290·4	13·5
4. Kamla	,, ,,			490	0·088	43·1	184	1·44	264·9	12·4
5. Kunda	,, ,,			68	0·240	16·3	150	1·70	255·0	10·9
Average ...					0·226					11·31

FIGURE.

Showing the relationship between the intake level and the per cent test dose return.



minimum requirement. It thus appeared that the minimum requirements level for Indian adults may not be less than 0·8 mg. (De, Mankad and Phatak, 1955, in press).

(*iv*) By carrying on subcutaneously injected test dose return experiment on a number of young girls with gradual increase of dietary level of riboflavin form 0·7 to 1·0 mg. per day, it has been observed that the minimum requirement of riboflavin for the girls may lie between 0·8 mg. and 0·9 mg. per day.

(*v*) Study of the effect of substitution of pulse protein by an equal amount of animal protein has shown that for equivalent amount of meat protein require more riboflavin for utilization than the pulse protein (De, Mankad and Datta, 1955, *Ind. Jour. Phys. Allied Sci.*, in press).

(*vi*) Investigation as to how far the high saturation dose could affect the blood level has revealed that in spite of saturation of the tissues by high saturation dose, the blood riboflavin was maintained at constant level by some hitherto unknown mechanism (De, Datta and Mankad, 1955, *Annal. Biochem. & Exper. Med.* in press).

During the remaining period of the year it is proposed to complete the work of estimation of the changes in riboflavin excretion during menstrual period and the estimation of minimum requirements of riboflavin for Indian boys between the age of 12 and 15 years.

THIAMINE METABOLISM WITH TYPICAL INDIAN DIETARIES.

As a part of the original scheme investigation on thiamine metabolism with Indian dietaries, an assessment of its minimum requirements for normal Indian adults has also been started in the year under review. The male subjects have been kept on a vegetarian rice and wheat and jowar diet and the experiment is being followed up in the same manner as was adopted in the study of riboflavin metabolism by the saturation and test dose technique. The subjects are at present passing through the saturation dose periods and the detailed results will be submitted in due course.

It is expected that by the end of March, 1956, some amount of work on thiamine metabolism with typical Indian dietaries will be completed.

On review of literature it has been observed that the incidence of toxæmia of pregnancy is related to thiamine deficiency (Siddal, 1938). Further Nixon *et al.* (1942) showed that in eclampsia the amount of thiamine excreted in urine was significantly lower than in normal subjects. It will be interesting to determine the level of thiamine in eclamptic subjects and to ascertain whether there is less synthesis of thiamine by intestinal flora and/or to what extent the additional thiamine administered to the patients is destroyed during this toxæmic condition.

It has also been reported (Seeler and Ott, 1944) that in malarial infection, riboflavin is selectively taken up by plasmodia causing minor avitaminosis symptoms. It will be interesting to investigate the minimum riboflavin level in blood which could prevent tissue anoxæa and yet will not increase the parasitæmia.

The help of Departments of Medicine and Gynæcology and Obstetrics of M. Y. Hospital, will be available to conduct the above work.

7. Studies on energy metabolism under Dr. S. Banerjee at the Presidency College, Calcutta.

In the previous year, from actual measurements of the surface area of the body of 15 normal subjects, it was found that the universally accepted formula of Du Bois for the calculation of the surface area of the body is not applicable in the case of Indians and a new formula—$\text{S.A.}(\text{cm}^2) = \text{H}^{0{\cdot}725}\ \text{cm.} \times \text{W}^{0{\cdot}425}\ \text{kg.} \times 74{\cdot}66$—was suggested. This year, to find out the normal BMR level of Indians under different environmental conditions, BMR and basal heat production in 24 hours was determined in 15 subjects, whose surface area was actually measured, during the summer seasons with the help of Collin's metabolex apparatus. The results are given in Table I.

It will be observed from Table I that BMR of all these subjects were less than the American or European standards. The BMR varied from —11·5 per cent to —27·0 per cent in comparison with the Mayo Foundation Standards for normal Americans. The total heat production in basal condition during 24 hours varied from 1,020 to 1,411 calories.

TABLE I.

Determination of BMR of normal subjects, whose surface areas of the bodies were actually determined, by Collin's metabolex apparatus during summer.

Name	Age, year.	Height, cm.	Weight, lb.	Basal heat production calories per 24 hours	BMR, per cent
D.B. ...	22	151·9	80·5	1,020	—16·5
S.L. ...	22½	153·9	115·8	1,174	—20·5
D.S. ...	24	157·8	105·5	1,097	—22·2
A.D. ...	44	157·4	144·3	1,262	—16·5
S.B. ...	19	160·5	104·5	1,164	—22·0
A.S. ...	20	161·2	98·8	1,080	—24·2
A.K.D. ...	21	160·6	90·5	1,068	—22·8
R.R.B. ...	27	162·7	97·0	1,147	—19·0
H.P.C. ...	28	164·4	102·8	1,162	—18·2
K.B. ...	19½	165·0	96·3	1,282	—11·5
C.C.D. ...	27	164·1	116·0	1,104	—27·0
A.G.R. ...	21	171·1	112·5	1,183	—25·0
D.C. ...	23½	171·9	120·8	1,344	—16·0
K.C. ...	23	172·1	127·0	1,411	—16·0
A.K.M. ...	30	177·1	142·0	1,267	—25·0

While BMR is determined with the metabolex apparatus, a R.Q. of 0·82 is assumed and the BMR is calculated from the heat equivalent per litre of oxygen at that R.Q. To find out whether the lower BMR obtained was due to the assumption of the R.Q. of 0·82 or to other causes, respiratory quotient of 34 students of this college was determined by the Douglas bag method.

Determination of respiratory quotient under basal condition.—The expired air of the subject for a definite period of time was collected in a Douglas bag. The oxygen, carbon dioxide and nitrogen contents of the air were determined with the Haldane's gas analysis apparatus. The atmospheric air was also analysed at the same time. The volume of the expired air was determined with a gasmeter. The volume of oxygen consumed and carbon dioxide liberated were calculated and respiratory quotient was obtained. The results are given in Table II:—

TABLE II.

Determination of R.Q. of normal male students under basal conditions by the Douglas bag method.

Name	Age, years	R.Q.
S.C.C.	15	0·825
D.B.	15	0·850
U.H.	15	0·740
N.S.	15	0·860
R.K.J.	16	0·975
S.K.D.	16	0·875
S.P.M.	16	0·800
S.M.	17	0·770
J.K.B.	17	0·855
K.G.	17	0·710
S.C.L.	17	0·890
G.B.	17	0·870
S.D.	17	0·950
A.K.B.	17½	0·920
P.S.C.	17½	0·835
R.N.B.	17½	0·825
S.D.	18	0·890
P.B.	18	0·980
B.K.B.	18	0·796
T.N.G.	18	0·935
D.G.	18	0·785
A.B.D.	18	0·765
B.A.	19	0·810
M.B.	19	0·910
R.C.D.	19	0·760
S.K.C.	19	0·820
T.K.M.	19½	0·800
B.S.	19½	0·800
C.R.D.	20	0·840
N.C.J.	20	0·865
A.B.S.	20	0·915
B.K.C.	20	0·770
M.L.D.	20	0·850
J.C.M.	20	0·855
Mean with Standard Error		0·843±0·014

It will be observed from Table II that R.Q. varied considerably from 0·71 to 0·98. The assumption of 0·82 as the R.Q. under basal condition is not desirable.

Determination of the basal metabolic rate in 34 students during the summer and rainy seasons by the Collin's metabolex and also by the Douglas bag method.— The basal metabolic rates of 34 students were determined in the summer and rainy seasons both with the Collin's metabolex and with the Douglas bag method. The room temperature varied between 84° F. and 90° F. The humidity varied from 70 per cent to 84 per cent. The students were fed a light meal consisting of milk and bread at 7 p.m. They slept for eight hours and in the morning the tests were performed at 8 a.m. before they took any food. Each student took rest in a recumbent position in the laboratory for 30 minutes before the actual tests were performed. They were accustomed to the experimental procedure adopted.

The oxygen consumption was determined with the metabolex apparatus in the usual way. From the oxygen consumption the total heat production in 24 hours under basal condition was calculated. By the determination of the surface area of the body by the use of our formula BMR in calories per square meter of the body surface per hour was calculated. The results are given in Table III.

TABLE III.

Determination of BMR and heat output in 24 hours in 24 normal students during summer and rainy seasons.

Age, years	With metabolex apparatus:		With the Douglas bag method:	
	Heat output in 24 hours, calories	BMR, calories per sq. m./hour	Heat output in 24 hours, calories	BMR, calories per sq. m./hour
15	1,386	37·56	1,535	41·64
15	1,325	35·19	1,386	36·82
15	1,282	30·30	1,477	34·93
15	1,118	34·43	1,190	36·63
16	1,038	30·09	1,266	36·76
16	1,323	33·55	1,409	35·75
16	1,319	36·05	1,341	36·70
17	1,326	37·89	1,370	39·18
17	1,256	31·03	1,561	38·60
17	1,227	33·16	1,439	38·89
17	1,136	29·93	1,440	38·05
17	1,439	39·18	1,355	36·91
17	1,196	30·83	1,324	33·33
17½	1,176	34·34	1,217	35·55
17½	1,277	33·99	1,290	34·35
17½	1,457	38·79	1,317	35·07
18	1,118	31·45	1,193	33·54
18	1,476	34·90	1,509	35·98
18	1,212	30·39	1,305	32·71
18	1,171	27·15	1,430	33·16
18	1,380	34·25	1,463	36·32
18	1,394	39·09	1,297	36·11
19	1,272	36·65	1,305	37·62
19	1,097	32·62	1,099	32·70
19	1.322	32·08	1,317	31·73
19	1,157	31·31	1,204	32·60
19½	1,301	31·14	1,305	31·25
19½	1,301	33·74	1,233	31·98
20	1,284	32·20	1,373	34·45
20	1,140	33·77	1,128	33·42
20	1,464	35·33	1,462	35·29
20	1,202	29·97	1,214	30·26
20	1,255	33·62	1,312	35·13
20	1,202	32·56	1,290	33·10

It will be seen from Table III that the total heat production under basal condition varied between 1,038 and 1,476 calories. BMR varied from 27 to 39 calories per square meter of the body-surface per hour. All these values were low in comparison with Western standards.

BMR was also determined by the Douglas bag method. Subject breathed ordinary air and the expired air was collected in the Douglas bag for 10 to 20 minutes. Oxygen, carbon dioxide and nitrogen contents of expired and inspired air were determined with Haldane's gas-analysis apparatus. The volume of the expired air was determined with gasmeter. R.Q. was determined and heat equivalent per litre of oxygen at the particular R.Q. was obtained from a table. From the volume of oxygen consumed, heat production in 24 hours was calculated under basal condition and BMR was also determined. The results are given in Table III.

The total heat production as determined by the Douglas bag method varied between 1,099 and 1,561 calories. The total heat production in most of the subjects as obtained by the Douglas bag method was higher than the value obtained with Collin's metabolex apparatus. BMR values obtained by the Douglas bag method varied between 30 and 41 calories per square meter of the body-surface per hour. The values although higher than those obtained with the metabolex apparatus were lower than the values obtained in Western countries.

The final evaluation of the normal BMR standards for Indians of different age groups and sex would entail a study with large number of subjects.

8. Inquiry on the rôle of folic acid and vitamin B_{12} in the metabolism of choline and methionine under Dr. A. Sreenivasan at the Department of Chemical Technology, University of Bombay.

(1) *Biosynthesis of choline.*—It was reported earlier that a net synthesis of choline from precursors by rat liver enzymes *in vitro* is not demonstrable because of the presence of the choline oxidase system (*Tech. Rep. S.A.B.*, 1954). Further work on the relationship of folic acid to the metabolism of choline was directed to a study of (*a*) serine metabolism, (*b*) phospholipid synthesis, and (*c*) choline synthesis in *Neurospora.*

(*a*) *Serine metabolism.*—Defined pathways of serine metabolism may be represented schematically as in page 106. Of these the reversible serine-glycine and serine-ethanolamine systems are of significance in transmethylation reactions. Although vitamin B_6 has been inferentially implicated in serine decarboxylation (*Jour. Biol. Chem.*, **201,** p. 299, 1953), it is now shown with rat liver enzymes that serine decarboxylation is not influenced by B_6 but is inhibited in folic acid deficiency or after aminopterin administration. The reverse reaction, viz., ethanolamine→ serine, involving carbon dioxide fixation, is influenced by biotin. It is also observed that folic acid and vitamin B_6 have both a functional significance in serine→ glycine conversion (Table I).

Serine: Pathways.

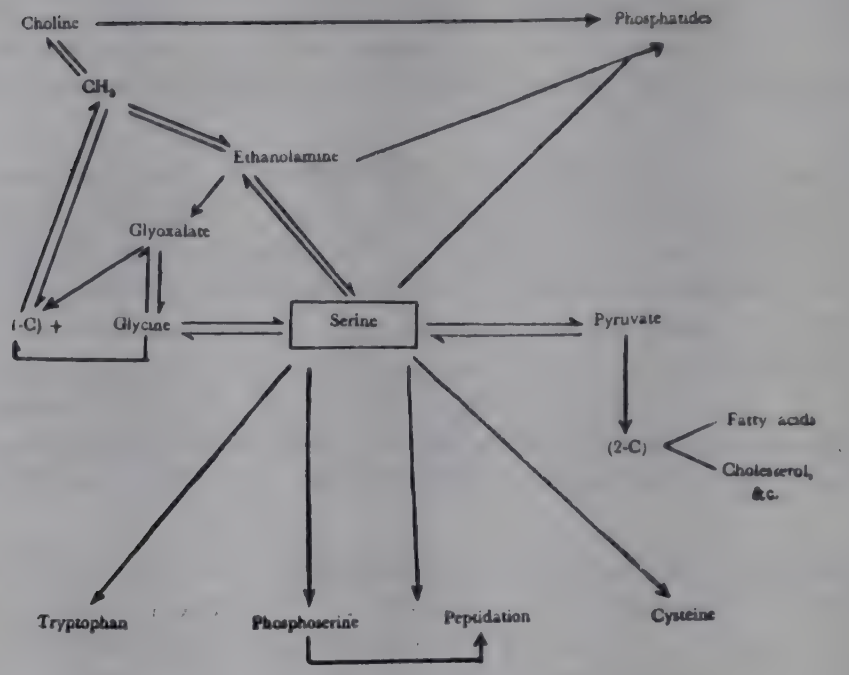

Choline
Phosphatides
CH_3
Ethanolamine
Glyoxalate
(1-C) +
Glycine
Serine
Pyruvate
(2-C)
Fatty acids
Cholesterol, &c.
Tryptophan
Phosphoserine
Peptidation
Cysteine

TABLE I.

Relationship of biotin, pyridoxine and folic acid to serine metabolism.

	Ethanolamine→ serine, per mg. liver N	SERINE DEHYDRASE		Serine decarboxylase	Glycine→ serine, per mg. liver N
		Pyruvate	NH_3		
		Per mg. dry weight			
	μM	μM	μM	μl	μM
—Biotin ...	0·89	0·48	0·47	53·9	...
+Biotin ...	1·74	0·58	0·59	50·2	...
—Pyridoxine ...	...	0·42	0·41	52·9	0·63
+Pyridoxine ...	...	0·51	0·51	52·0	1·14
—Folic acid ...	...	...	0·50	34·4	0·72
+Folic acid ...	...	...	0·52	52·6	1·37

Serine dehydrase activity has been followed in the foregoing studies and is found to be influenced by both biotin and B_6 but not by folic acid.

In view of the recent increased interest in single carbon addition reactions (*Biochem. Jour.*, **58,** p. 448, 1954; *Jour. Biol. Chem.*, **214,** pp. 47, 821, 1955), a more detailed study of the serine → glycine conversion has been planned.

(*b*) *Phospholipid synthesis.*—It was reported earlier that, in forced methylation as well as in a folic acid deficiency, there is an increase in neutral lipids and a decrease in phospholipids of the liver (*Biochem. Jour.*, **59,** p. 470, 1955). A relationship of folic acid to phsopholipid synthesis is also inferable from the well-known effect of this vitamin on choline metabolism. However, attempts to follow up phospholipid synthesis *in vitro* with whole liver homogenates or mitochondria have not hitherto been successful. The systems studied included various combinations of the following additions: Succinate, ATP, Mg^{++}, F^-, besides choline, a monoglyceride or a fatty acid. With mitochondrial preparations, glycerol and glycerokinase (of supernatant) additions (*Jour. Biol. Chem.*, **211,** 951, 1954) were also tried. The reported incorporation of choline into phospholipid by mitochondria (*Jour. Biol. Chem.*, **209,** p. 525, 1954) is presumably, therefore, an exchange reaction.

The probable precursorial rôle of phosphoryl-choline in phospholipid synthesis was indicated last year. A method has been worked out for the assay of tissue phosphoryl choline. The influence of dietary folic acid on phosphoryl choline content of rat tissues will now be studied (*Bioch. Jour.*, **60,** p. 325, 1955).

(*c*) *Choline synthesis in* Neurospora crassa (*cholineless*).—The preformed mycelium of *Neurospora crassa* (cholineless) could utilize methionine and ethanolamine for the biosynthesis of choline (Table II). Serine could substitute for ethanolamine, but less effectively, on account of the limiting activity of serine decarboxylase. The organism does not have a choline oxidase system, all the choline synthesized being accounted for as phospholipids. Respiring cells of the organism incorporate added choline quantitatively into phospholipids. The influence of folic acid and vitamin B_{12} on this system is being ascertained.

TABLE II.

Utilization of choline precursors by Neurospora crassa (*cholineless*).

	Additions to medium (1·0 mg. /10 c.c.)	Mycelial dry weight (mg.)	Per cent increase in dry weight	Total choline (γ/100 mg. dry weight)
1	*Nil* ...	16·9	...	153
2	dl-ethanolamine ...	19·8	17·1	147
3	dl-methionine ...	22·3	31·9	149
4	dl-Serine ...	17·0	...	141
5	As in 2+3 ...	29·1	72·1	156
6	As in 3+4 ...	25·7	52·0	150
7	As in 2+3+4 ...	30·3	82·8	140
8	As in 2+4 ...	19·2	13·6	149

(2) *Biosynthesis of adrenaline.*—It was reported last year that folic acid influences adrenaline biogenesis in a manner similar to its mediation, indirectly, in the methylation of glycocyamine and nicotinamide (*Biochem. Jour.*, **59,** p. 470, 1955). This effect of folic acid has now been ascertained further from studies on the reversible glucose → glycogen transformation in the isolated rat diaphragm and using aminopterin-fed and control animals, with and without added adrenaline and insulin (Table III). Other observations with the rat diaphragm have shown that aminopterin inhibits not merely glycogenolysis but also hexokinase action.

TABLE III.

Effect of aminopterin on the metabolism of the rat diaphragm.

Additions	Initial glycogen mg./g. of diaphragm	Glycogenesis	Glucose disappearance
		Mg./g. of diaphragm during 2 hours	
Normal animals			
None ...	3·7	0·38	3·2
+Adrenaline ...	2·7	0·15	3·0
+Insulin ...	3·7	0·87	5·7
Aminopterin-fed animals			
None ...	5·2	0·65	5·4
+Adrenaline ...	5·1	0·57	4·0
+Insulin ...	5·2	0·71	6·3

(3) *Vitamin B_{12} and oxidative metabolism.*—It was reported earlier that in thyrotoxicosis there is a marked reduction in conjugating ability of liver enzymes (*Nature*, **176,** p. 165, 1955). Vitamin B_{12} supplementation counteracts this condition appreciably through more effective coupling of exergonic with endergonic processes. That the primary manifestation of hyperthyroidism is a B_{12} deficiency and consequent interference in the formation and utilization of sulphydryl enzymes was further ascertained by a study of the rate of incorporation of administered cysteine into liver sulphydryl compounds (Table IV).

TABLE IV.

Sulphydryl levels in the hyperthyroid rat.

Group	WITHOUT CYSTEINE: Liver	WITHOUT CYSTEINE: Blood	WITH CYSTEINE*: Liver	WITH CYSTEINE*: Blood
	Mg. per: g.	Mg. per: 100 c.c.	Mg. per: g.	Mg. per: 100 c.c.
Control				
B_{12}-deficient ...	0·75	10·94	2·13	23·96
B_{12}- fed	0·83	11·48	3·59	23·17
Hyperthyroid				
B_{12}-deficient ...	0·27	25·63	0·96	39·17
B_{12}-fed	0·57	18·75	2·09	27·60

* 30 mg. cysteine given as a single dose intraperitoneally 3 hours prior.

A similar explanation is tenable in respect of vitamin B_{12} protection of hepatic injury by carbon-tetrachloride. As reported by Patwardhan and co-workers (*Ind. Jour. Med. Sci.*, **7**, p. 533, 1953), a marked reduction in tissue (liver) sulphydryl precedes all other effects of carbon tetrachloride toxicity, viz., increased lipids, lowered glycogen and ribose nucleic acid (Table V) and an enhanced activity of choline and xanthine oxidases, among others. Vitamin B_{12} corrects these derangements, restoration of glycogen stores in liver being the slowest. The observations include study of centrifugally separated cell fractions.

TABLE V.

Vitamin B_{12} and CCl_4-injury.

	Total lipids	RNA percentages	SH compounds	Glycogen
48 *hours*				
Stock diet ...	7·9	3·6	0·13	0·36
$+CCl_4$	16·1	2·3	0·12	0·07
$+B_{12}$	7·5	3·8	0·18	0·38
$+B_{12}+CCl_4$ ...	11·5	3·4	0·17	0·06
Six hours				
Stock diet ...	7·8	3·5	0·14	0·33
$+CCl_4$	9·6	2·9	0·06	0·10
$+B_{12}$	7·5	3·8	0·16	0·34
$+B_{12}+CCl_4$ ...	9·3	3·1	0·13	0·15

Study of the intracellular distribution of sulphydryl compounds has revealed that the mitochondria are completely devoid of this metabolite and that the small amount present in nuclei is relatively less labile under conditions of nutritional stress (Table VI). A paper electrophoretic procedure for glutathione assay has been completed preliminary to studies on the biosynthesis of glutathione by liver enzymes in relation to folic acid and viatmin B_{12}.

TABLE VI.

Distribution of SH compounds in liver.

	Normal	CCl_4	Normal	Hyperthyroid
	Mg./g. of liver			
Whole liver	116·7	40·7	83·0	57·3
Nuclei	16·6	16·6	12·0	8·9
Supernatant	99·5	23·7	72·1	49·2

9. Investigation on the influence of cooking on the nutritional value of foods under Dr. M. L. Pai at the Medical College, Baroda.

The amount of work done in India on the influence of cooking on the nutritional value of foods prepared from cereals, etc., such as, wheat and others, is much too little compared to the detailed investigations made on rice, in spite of the fact that apart from rice, wheat, etc., also form important cereals in Indian diets (Patwardhan, 'Nutrition in India', 1952). The chemical analysis of the raw articles of food may sometimes be misleading as a guide to its nutritional value, because some of the material estimated in the raw articles may not be available for nutrition after it has been subjected to the varied changes during the process of cooking. The cause for the lack of work on cereals, etc., such as, wheat and others as compared to that on rice may perhaps be attributed to the fact that these food-grains are very much less subjected to processing as compared to rice (Patwardhan, *loc. cit.*). The analysis with respect to the estimations of nitotinic acid, riboflavin, thiamine, ascorbic acid, vitamin A (when present) and those of calcium, phosphorus and iron were carried out in half the portion of the sample of the raw material and then in the remaining half of the portion which is cooked. In the report submitted last year for the work done on this inquiry during the period from April 1954 to September 1954, the results of the analysis of ten items of the articles of food, such as 'chapati', 'bhakari', 'puri', etc., prepared from wheat, 'jowar', 'bajra', etc., were given.

During the time under report, i.e., from October 1954 to September, 1955, the above ten items were further analysed for the remaining estimations like those of nicotinic acid, which were not done and hence not reported then and the results of analysis of these ten times are given in Table I. Besides these, the following items have been studied and shown in Table II.

TABLE I.

Nicotinic-acid content of the food articles per 100 *g. of dry weight.*

Serial number	Article of food	Nicotinic acid, mg.	Serial number	Article of food	Nicotinic acid, mg.
1.	Dough of 'chapati' from wheat flour ...	3·95	6.	'Puri' from white wheat flour ...	2·90
2.	'Chapati' from wheat flour ...	3·36	7.	Dough of 'bhakari' 'jowar' flour (white)...	3·46
3.	Dough of bread (bakery, white) ...	3·63	8.	'Bhakari' from 'jowar' flour (white) ...	3·09
4.	Bread (bakery white)	3·13	9.	Dough of 'bhakari' from 'bajra' flour ...	3·17
5.	Dough of 'puri' from white flour ...	3·27	10.	Bhakari from 'bajra' flour ...	2·92

Note.—The samples were run in duplicate and the above are the average values.

TABLE II.

Nutritive value of foods—average composition per 100 g. of dry weight.

Sample number	Preparation	Moisture g.	Ash g.	Carotene (Vitamin A I.U.)	Thiamine, μg.	Riboflavin, μg.	Nicotinic acid, mg.	Ascorbic acid, mg.	Phos-phorus, mg.	Iron, mg.	Calcium, mg.
1	2	3	4	5	6	7	8	9	10	11	12
1.	Dough unfermented for preparing ‘ Idli ’	67·51	4·32	...	399·0	128·0	3·02	...	279·3	7·35	80·10
2.	Dough fermented for preparing ‘ Idli ’ ...	69·16	4·83	...	216·0	126·0	3·23	...	310·8	8·11	78·10
3.	‘ Idli ’	66·70	4·50	...	176·0	109·0	3·05	...	307·5	7·01	83·02
	Raw materials (calculated) (namely, rice: black gram :: 4:1)	...	0·75	13	135·0	...	1·5	...	170·0	3·0	50·0
4.	Dough unfermented for preparing ‘ Dosa ’	66·13	4·15	...	348·0	119·0	2·91	...	268·1	8·13	85·03
5.	Dough fermented for preparing ‘ Dosa ’	67·78	3·95	...	212·0	115·0	3·02	...	247·0	7·90	82·30
6.	‘ Dosa ’	34·48	2·45	...	147·0	68·0	2·20	...	201·0	7·05	69·10
	Raw materials (calculated) (namely, rice: black gram :: 4:1)	...	0·75	13	135·0	...	1·5	...	170·0	3·0	50·0
7.	Cauliflower (raw) on wet weight basis ...	90·02	1·43	45	270·0	99·0	0·88	63·25	55·6	1·5	39·2
8.	Cauliflower made ready with ingredients added for preparing ‘ bhaji ’ (pre-cooked)	81·43	2·83	230	510·0	317·0	2·91	281·0	192·0	4·24	143·0
9.	Cauliflower ‘ bhaji ’ (cooked) ...	47·99	2·49	151	285·0	193·0	2·10	69·2	201·0	4·01	129·8
	Raw cauliflower (referred)	89·4	1·4	38	330·0	80·0	0·9	66·0	60·0	1·30	30·0
10.	Cabbage (raw) on wet weight basis ...	91·0	0·78	1,605	121·0	45·0	0·57	114·0	40·0	1·17	37·8
11.	Cabbage made ready with ingredients added for preparing ‘ bhaji ’ (pre-cooked) ...	84·9	2·2	2,960	271·0	160·0	1·9	410·0	221·0	4·12	128·1

TABLE II.—(*Contd.*)

Sample number	Preparation	Moisture g.	Ash g.	Carotene (Vitamin A I.U.)	Thiamine, μg.	Riboflavin, μg.	Nicotinic acid, mg.	Ascorbic acid, mg.	Phosphorus, mg.	Iron, mg.	Calcium, mg.
1	2	3	4	5	6	7	8	9	10	11	12
12.	Cabbage ‘ bhaji ’ (cooked) … …	64·25	2·01	1,789	144·0	88·2	0·93	118·4	230·0	4·46	119·0
	Raw cabbage (referred) … …	90·2	0·6	2,000	150·0	30·0	0·4	124·0	50·0	0·8	30·0
13.	Rice after being washed (pre-cooked) …	32·7	0·67	…	42·0	45·0	2·0	…	176·2	1·6	18·1
14.	Rice (cooked) … … …	74·7	0·47	…	25·0	28·0	1·7	…	163·0	1·7	15·5
	Rice raw milled (referred) … …	13·0	0·4	0	60·0	80·0	1·2	…	110·0	1·0	10·0
15.	‘ Dahl-tuver ’ made ready with ingredients added for preparing liquid ‘ dahl ’ (pre-cooked) … … …	39·6	7·6	559	484·0	189·0	2·27	4·1	252·0	5·2	82·0
16.	‘ Dahl-tuver ’ (liquid) (cooked) …	72·01	7·42	266	333·0	167·0	1·9	1·95	237·0	5·6	92·1
	(Tuver) Red gram dahl (referred) …	15·2	3·6	220	450·0	…	2·4	…	260·0	8·8	140·0
17.	‘ Mung-dahl ’ made ready with ingredients added for preparing liquid ‘ dahl ’ (pre-cooked) … … …	32·5	6·9	487	421·0	181·0	1·96	3·91	270·0	6·1	93·0
18.	‘ Mung-dahl ’ (liquid) (cooked) …	80·2	7·1	210	349·0	162·0	1·7	1·2	280·0	6·6	84·1
	Green gram ‘mung’ without husk (referred)	10·4	3·6	158	465	…	2·0	…	280·0	8·4	140·0
19.	‘ Khichdi ’ of rice and ‘ tuver-dahl ’ made ready with ingredients added (pre-cooked)	32·63	1·93	1,987	219·5	163·0	2·8	3·01	207·0	5·1	71·2
20.	—do.— (cooked)	76·34	1·73	1,394	139·4	117·0	2·1	1·6	193·0	4·8	63·1
	Raw materials (calculated) namely rice: ‘ tuver-dahl ’ :: 8:3 … …	…	1·3	…	166·0	…	1·5	…	150·0	3·1	45·0

TABLE II—(*Concld.*)

Sample number	Preparation	Moisture g.	Ash g.	Carotene (Vitamin A I.U.)	Thiamine, μg.	Riboflavin, μg.	Nicotinic acid, mg.	Ascorbic acid, mg.	Phos-phorus, mg.	Iron, mg.	Calcium, mg.
1	2	3	4	5	6	7	8	9	10	11	12
21.	‘Khichdi’ of ‘Kodri’ and ‘tuver-dahl’ made ready with ingredients added (pre-cooked) … … …	32·5	1·73	1,702	277·1	91·0	2·3	2·87	189·0	4·9	71·1
22.	—do.— (cooked)	71·94	1·51	1,517	155·4	78·1	1·9	1·01	190·0	5·02	68·2
	Raw materials (Kodri: Liver-dahl :: 8:3) (calculated) … … …	…	1·2	…	158·0	…	1·2	…	127·0	3·2	46·0
23.	‘Kodri’ (pre-cooked) … …	30·61	0·46	0	54·15	42·7	1·3	0·26	105·0	1·8	16·3
24.	‘Kodri’ (cooked) … …	78·12	0·37	0	37·2	36·1	1·02	0·08	101·4	1·93	15·9
25.	Raw ‘Kodri’ on wet weight basis …	…	0·32	0	48·0	34·7	0·8	0·18	69·9	1·2	11·0
26.	‘Kovai’ fruit tender ‘Tondle’ (pre-cooked)	89·9	6·4	1,989	438.0	418·0	18·9	167·0	195·0	16·8	213·0
27.	—do.— (cooked)	85·02	6·5	1,697	316.0	337	14·2	35·4	157·5	15·7	218·0
	‘Kovai’ fruit tender (referred) …	93·1	0·5	260	…	…	…	28	30	1·4	40·0
28.	Pumpkin—‘bhaji’ (pre-cooked) …	93·3	8·2	985	598·0	402·0	8·4	21·4	187·0	9·1	63·2
29.	—do— (cooked) …	92·0	8·05	920	493·0	341·0	7·1	4·9	160·0	8·9	65·1
	Pumpkin (raw) (referred) … …	92·6	0·6	84	60·0	40·0	0·5	2·0	30·0	0·7	10·0
30.	Potato—‘bhaji’ (pre-cooked) …	68·98	3·4	642	310·0	67·5	6·1	44·7	101·5	2·9	36·6
31.	—do.— (cooked) …	54·54	3·6	560	189·0	55·2	5·7	16·5	110·2	3·1	38·0
	Potato (raw) (referred) … …	74·7	0·6	40	60·0	10·0	1·2	17·0	30·0	0·7	10·0

The samples were run in duplicate for analysis and the above are the average values.

(1) *Unfermented dough for the preparation of ' Idli '.*—This is prepared by taking rice in the form of small granules and ' dahl ' of black gram, in proportions of about 4:1 which mixture is then kept in water for some time. These are then pounded into very small granular form and then by adding a small amount of water to it, the whole mass is now made homogneous and to it a little salt is added and mixed.

(2) *Fermented dough for the preparation of ' Idli '.*—The above mixture is allowed to ferment by keeping it in a large vessel overnight.

(3) *' Idli '.*—Small portions from the fermented dough, obtained above, are cooked in steam till the granules are made soft.

(4) & (5) *Unfermented & fermented dough for the preparation of ' Dosa '.*—This preparation is very much similar to that made for the preparation of ' Idli ' described in items No. 1 and 2 except for the fact that in the present case, the mass is made into a very much finer particulate form than in the case of the former and this is then allowed to ferment.

(6) *' Dosa '.*—Small portions from the above fermented dough are taken and fried by taking a little quantity of oil on a shallow pan on open fire, after covering the pan with a shallow vessel.

In case of the items in numbers from 7 to 12, these vegetables, namely, cauliflower and cabbage, were first analysed in their raw forms, i.e., as they are obtained from the market. Then these vegetables, after the edible portion having been cut into small pieces, as is the usual procedure adopted by the housewives for preparing ' bhaji ' out of them, were washed and to them were added other ingredients, such as salt, turmeric, oil, etc., and were thoroughly mixed. One-half of the portion was used for the analysis for studying the composition of uncooked material while the other half of the portion was cooked till it was soft and was made into the form of ' bhaji '. This cooked preparation of ' bhaji ' was then analysed.

In case of the preparations in numbers from 13 to 18, these were the various items that usually go to form a dish commonly taken by the people on this side of the country, for one of their meals of the day, usually the afternoon meal.

The average weights of the various constituents that go to form such a single dish served to an adult and the average weights of the raw materials used for preparing those constituents are given in Table III.

TABLE III.

Average weights of the various constituents of a single meal served to an adult.

Serial number	Preparation	Total weight, g.	Average weight, g.
1.	Rice ...	260	...
2.	' Chapati ' (No. 5) ...	176	35 (of a single ' chapati ').
3.	Liquid ' dahl ' (tuver) ...	329	...
4.	Vegetable ...	60	...

An average weight of a ' puri ' which is taken as a substitute for a ' chapati '...13·1 g.

Average weights of the raw ingredients taken to prepare the above constituents.

Number	Name of the article and of the preparation made from the former	Total weight, g.	Average weight, g.
1.	Raw rice for preparing cooked rice	116·2	
2.	Wheat flour for preparing 'chapati' (No. 5)	143·9	28·8 g. of wheat flour.

In the present case, the rice is prepared by the usual process of first having washed the granules with sufficient water, two or three times, and then cooked in a vessel on open fire, by taking about double the amount of water for cooking. When the grains are made soft, this water used for cooking is thrown away.

The ' dahl ' from ' mung ', or from ' tuver ' are the liquid forms in which these pulses are consumed by the people. These are prepared by first washing, with water, twice or thrice, the granules of the ' dahls ' of these pulses, which are obtained by grinding the pulses into two parts from which the husks are removed. Then to these, salt, chillies, turmeric, etc., are added and well mixed. It is then cooked in about double the quantity of water till the grains are made soft.

On many occasions, the dish known as ' khichdi ' (*vide* item No. 19 and onwards in Table II) forms one of the major items or perhaps the only item of one of the meals, of the day, taken by the people on this side. ' Khichdi ' made up of rice and ' tuver dahl ' is prepared by taking these two ingredients in proportion of 8:3, mixing and washing with water, twice or thrice. A little salt, turmeric, ghee, etc., are added and after mixing, it is cooked in about equal quantity of water, till the grains are rendered soft.

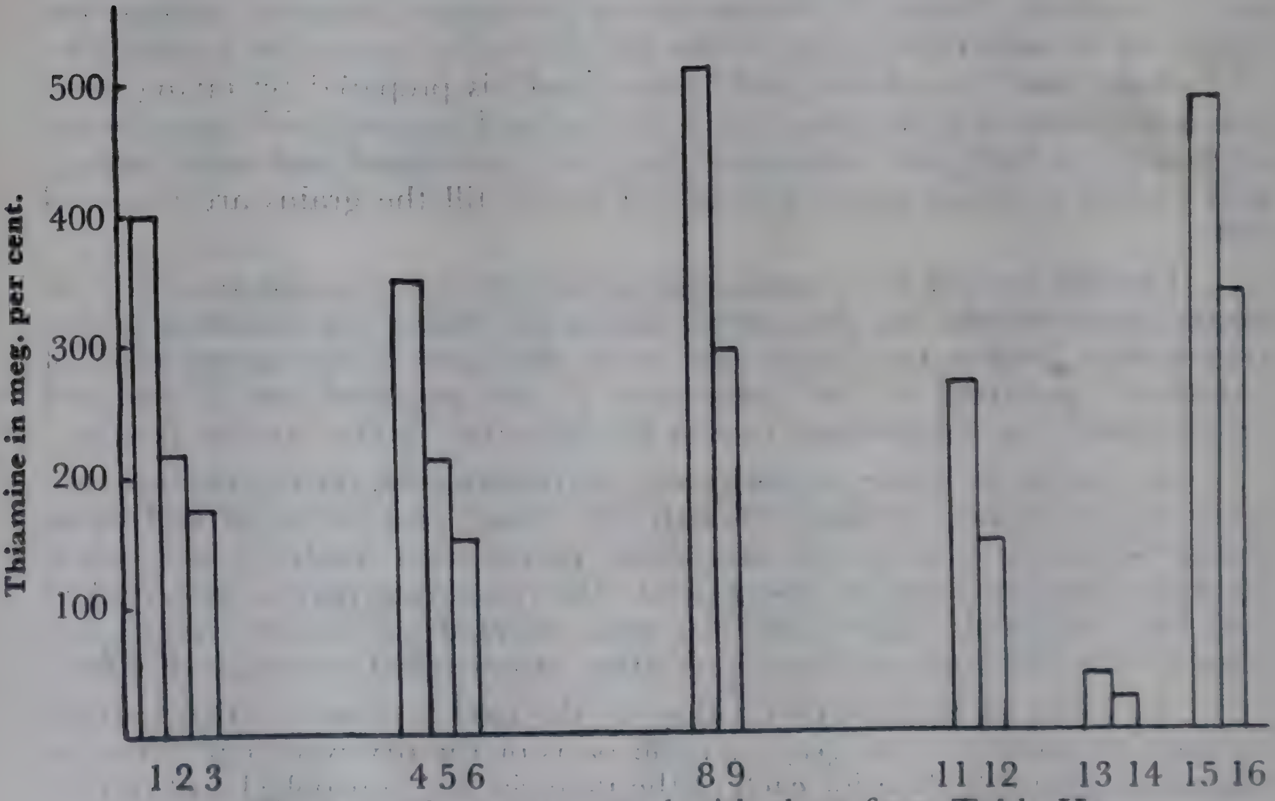
On this part of the country, the people belonging to the poor-income groups occasionally eat the cereal known as ' kodri ' in the form of the preparation similar to cooked rice or in the form of the preparation of ' khichdi ' prepared in the same way as one prepared out of rice and ' tuver-dahl ', by substituting rice in the latter by ' kodri ' in the former.

In case of the above preparations, the condiments, such as chillies, etc., were added before cooking, though the usual practice is to add them during cooking, because the uncooked preparation analysed was taken from the mixture made as above while the remaining portion was cooked and then analysed. The procedure thus adopted gave more comparable data for the difference in the composition of uncooked and cooked items.

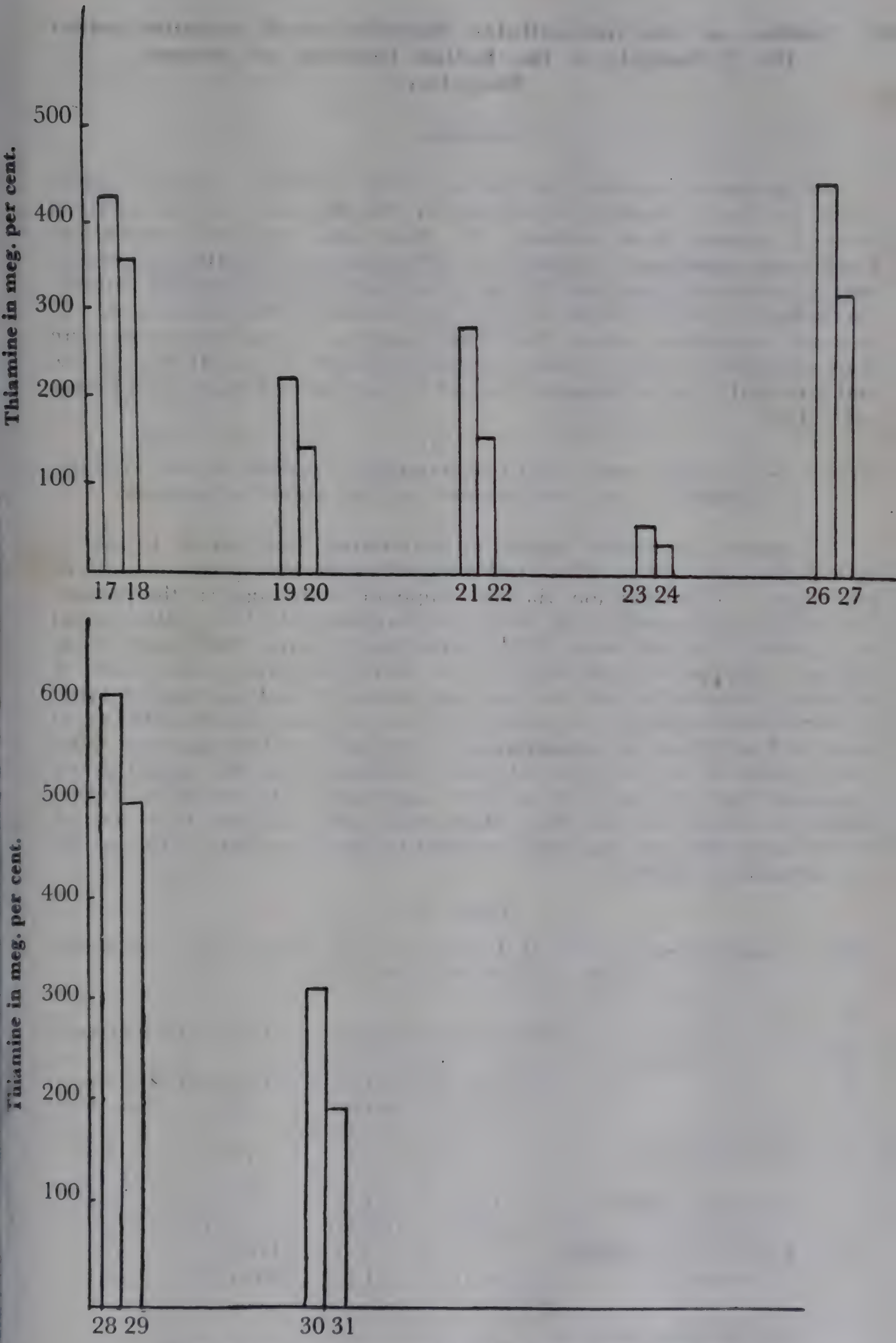
In Table II the nutritive values of the food articles for their various constituents analysed are given on the basis of the dry weight in order to have a comparable data for each of the items in their uncooked and cooked preparations, respectively.

In the same Table, the respective nutritive value and chemical composition of each of the items, as calculated on the basis of the raw materials which were used for preparing that item are given. These are taken from the health bulletin. Of course, in these calculations the raw materials considered are the basic ones, i.e., without taking into consideration the various ingredients, such as condiments, spices, etc., added during the preparation of the item. These calculated values are, however, based not on the dry weight, but on the basis of the item being in its original form, i.e., on the wet weight basis (Table II).

As can be seen from the Table as also from the Graphs there is a significant loss of the vitamins studied, particularly that of the thiamine and vitamin C, to a much larger extent than that of the riboflavin or nicotinic acid or vitamin A when the articles of the foodstuffs are subjected to the different procedures of cooking. The loss of the thiamine and vitamin C is observed quite significantly even when the articles of the foodstuffs are washed several times before they are cooked, as that in the case of the vegetables or rice, etc., and when their nutritive values are compared with those of unwashed, i.e., raw articles. The results also show that the loss of the vitamins studied in these preparations which have been subjected to the prolonged heating or boiling, etc., at high temperature are more than those in the cases of preparations where they have not been subjected to such prolonged heating, etc., at high temperature as the process of baking, etc., as in the case of the preparations of 'chapati', 'bhakari', prepared from cereals, such as wheat, 'jowar', 'bajra', etc.



These numbers correspond with those from Table II.



These numbers correspond with those from Table II.

10. Studies on the intracellular distribution of vitamins under Dr. J. Ganguly at the Indian Institute of Science, Bangalore.

SYNOPSIS.

Experiments involving the blocking of the reticulo-endothelial system of the rat show a marked interference in the deposition of the esterified form of vitamin A in rat-liver, the free form remained unaffected. Preliminary experiments consisting of differential centrifugation of chicken liver homogenates show that the carotenoids are mostly associated with the particulate matter, while the vitamin A is present in the supernatant. In separate experiments, where liver homogenates of normal stock rats were dialysed against a series of buffers ranging from pH 7·0 to pH 4·0, the free and esterified forms of vitamin A could be precipitated down at different pH values.

EFFECT OF BLOCKING THE RETICULO-ENDOTHELIAL SYSTEM ON THE STORAGE OF VITAMIN A ESTER AND ALCOHOL IN THE LIVER OF THE RAT.

Vitamin A is absorbed mostly in the esterified form via the lymphatic system through systemic blood and is stored in the liver predominantly in ester form. Its absorption via the lymphatic route suggests the possible rôle of the Kupffer cells in its storage mechanism in the liver. Rats raised on vitamin A low diet were divided into three groups. One group of six rats was kept on the basal diet alone to serve as negative control, each of a second group of six rats received one intraperitoneal injection of 1 ml. of normal saline daily for seven days and a single oral dose of 3,000 μg. of vitamin A in 0·2 c.c. of groundnut oil on the last day of the injection and a third group of six rats received similar treatment as the second group excepting that 1 c.c. of a 20 per cent suspension of Indian ink in normal saline, instead of normal saline alone, was used. All rats were starved for 24 hours, after the dose and sacrificed by heart puncture. The results are presented in Table I.

TABLE I.

Effect of intraperitoneal injection of Indian ink on the vitamin A ester and alcohol values in blood and liver of rats.

Number of rats	Treatment	BLOOD PLASMA (μG./C.C.)		LIVER (μG./ORGAN)	
		Vitamin A ester	Vitamin A alcohol	Vitamin A ester	Vitamin A alcohol
6	Negative control ...	*	*	10·2 (8-12)	1·4 (1-1·8)
6	Saline *plus* 3,000 μg. Vitamin A ...	1·9 (1·6-2·2)	1·2 (0·8-2·0)	2238·3 (1980-2450)	56·0 (45-65)
6	India ink *plus* 3,000 μg. Vitamin A ...	5·1 (3·5-8·0)	1·9 (1-2·5)	1168·8 (900-1322)	54·3 (47-61)

* Too low for determination.

The above results show that R.E.S. blocking markedly interferes with the storage in the liver of the ester form of vitamin A, the alcohol form remaining unaffected.

In separate experiments, where liver homogenates of normal stock rats were dialysed against a series of buffers ranging from pH 7·0 to pH 4·0, the free and esterified forms of vitamin A could be precipitated down at different pH values.

11. Studies on metabolism of starvation and malnutrition under Professor M. L. Chakraborthy at the Medical College, Calcutta.

Materials.—Subjects of malnutrition, number of cases 30 (both children and adults).

Average body-weight.—About 40 per cent less than the expected body-weight.

Experimental details.—By standard experimental methods.

The following are the results of investigation.

Average general condition of the patients.—Comparative sense of well-being, no gross sign of avitaminosis. The patients with total protein below 4·5 per cent developed œdema. Some of these cases are being followed up to find out the results after test diets. These cases will be reported later on.

Though the present work should have been limited to mineral metabolism, hæmatological and biochemical examinations were thought a necessity to find out whether alterations in the mineral contents of blood can be correlated with corresponding changes in the hæmatology and biochemistry of blood. These investigations will also give some data which can be compared with those obtained during the past investigation including the present author's observation of the past Bengal famine. This will help in stamping out genuine cases of malnutrition from the present series of investigation.

BLOOD

HÆMATOLOGICAL

	Maximum	Minimum	Average
R.b.c. ...	4 m/cmm.	1·5 m. /cmm.	3·5 m/cmm.
W.b.c. ...	6,000/cmm.	4,000/cmm.	4,500/cmm.
	Per cent	Per cent	Per cent
Neutro ...	60	50	58
Lympho ...	35	25	32
Mono ...	5	2	3
Eosino ...	6	2	3
Baso ...	1	*Nil*	*Nil*
Hb. per cent	10g.	5g.	7g.
P.C.V. ...	45	30	40
M.C.V. ...	87CM*	100CM.	65·7CM.
M.c.h. ...	25YY	33·33YY	20YY

* Cubic micron.

BIOCHEMICAL

	Maximum	Minimum	Average
	Per cent	Per cent	Per cent
Sugar ...	90 mg.	55 mg.	65 mg.
Cholesterol ...	110 ,,	90 ,,	95 ,,
Total protein ...	6	4	5
Urea ...	32 mg.	20 mg.	24 mg.
N.P.N. ...	30 ,,	18 ,,	20 ,,
Creatinine ...	2 ,,	1 ,,	1·5 ,,
Sodium ...	325 ,,	315 ,,	318 ,,
Potassium ...	18 ,,	14 ,,	15 ,,
Calcium ...	11 ,,	19 ,,	10 ,,
Magnesium ...	1·5 ,,	1 ,,	1 ,,

URINE

	Per cent	Per cent	Per cent
Chloride ...	0·6	0·5	5·2
Urea ...	0·8	0·6	0·7

Reaction—acid (in most cases.)
Sugar, acetone, albumen, blood and bile—*Nil.*
Microscopic examination—nothing noteworthy.

LEPROSY.

1. Leprosy inquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta.

It has been possible to develop and standardize electron and phase microscopic methods, some of which provide satisfactory pictures of the internal structures and others of the surface of the bacilli. Using these methods, several different morphological forms of the leprosy bacillus have been encountered. Comparable forms have been seen in materials from the same source with the phase microscopy and by the usual Ziehl-Neelsen method of staining. It is, therefore, concluded that the observed forms represent various characteristic features of leprosy bacillus and are not the result of any particular technique.

Intracellular lipoids have been found only in cases of the lepromatous type, in the foam cells or in vacuolated histiocytes. Vacuolated histiocytes or epitheloid cells are sometimes encountered in lesions from tuberculoid cases, specially during the phase of reaction; these vacuoles, however, do not contain any lipoid material and are probably of hydropic nature, due to œdema of the tissue. The presence of intracellular lipoids is thus an additional help in differentiating true lepromatous lesions from other similar looking lesions.

The lepromin test has been found to be of great prognostic value in contacts, a positive result indicating a good prognosis. Compared to the positive reactors, the negative reactors are not only more likely to get the disease, but are also more likely to get the serious form of the disease.

Hydno-sulphone, a condensation product of hydnocarpic acid and DDS, has been found to be effective and well tolerated. It is specially useful in cases which are intolerant to DDS. Dihydroxy-DDS, a nuclear hydroxy derivative of DDS, gave very encouraging results in the beginning, but later was found to be unsatisfactory. Combined use of INH and DDS has produced satisfactory results, and it is possible that the development of drug resistance to INH is considerably delayed by the addition of DDS. Results with the combined treatment appear to be better than those with DDS alone. The indigenous drug tested during the year did not produce any clinical or bacteriological improvement.

1. Electron and phase microscopy of myco-lepræ.

Attempts have been made to establish with certainty whether the various forms of leprosy bacilli observed are merely due to effects of techniques in the preparation or whether they represent the different morphological characteristics of the leprosy bacillus.

The following forms were encountered during electron microscopy: (*a*) Oval-shaped bodies with one or double condensations with lengths varying from 0·8 to 1·6 μ, (*b*) elongated types, approximate length 2·2 μ with double polar condensations, (*c*) elongated types (3·0 μ or more in length) with alternate zones of light and dark regions, (*d*) homogeneously dense types either of uniform diameter throughout the length or of wider diameter in the middle of the bacilli with tapering ends; and (*e*) bacilli in the process of budding, branching and dividing. In addition to the usually described lateral budding, terminal budding from one end of the bacillus has also been noticed.

Comparable forms were seen with phase microscopy of living bacilli and routine microscopy of the stained bacilli. This would very strongly indicate that the various forms observed are not the results of preparation techniques, but that they represent the various characteristic features of the leprosy bacillus itself. Further, the fact that all these forms have been observed in the same individual suffering from active disease previously untreated, rules out the possibility that some of these forms could have been the result of treatment. It is, however, possible and likely that some of the observed forms may be encountered more frequently in patients under treatment than otherwise; this matter is under study. It is likely that the observed variants represent different stages in the development of the leprosy bacillus under different conditions. Investigations are in progress to get definite information on the subject.

A paper giving details of this investigation will shortly be appearing in the *International Journal of Leprosy.*

2. INTRACELLULAR LIPOID IN BIOPSY SPECIMENS FROM CASES OF LEPROSY OF DIFFERENT TYPES.

An interim report was presented last year on the result of examination of biopsy specimens from 45 cases of leprosy of different types. It was concluded that the presence of coarse orange coloured granules in sections stained with Sudan III offered an additional evidence regarding the lepromatous nature of the lesion.

The study has been continued during the year under report and biopsy specimens were examined from another 46 cases of leprosy of different types and from two cases not suffering from leprosy. The classification of these cases, based on clinical, bacteriological, histological (routine paraffin section) and lepromin test, was as follows: Lepromatous 21, possibly lepromatous 1, borderline 2, tuberculoid 20 of which 7 were reacting (of these three were bacteriologically positive) and 13 quiescent, and maculoanæsthetic 2. The findings regarding the presence or absence of intracellular lipoids in the above groups are summarized in Table I:—

TABLE I.

Type of cases	Total number examined	FINDING WITH RELATION TO THE PRESENCE OF LIPOID:			
		Orange coloured coarse granules ++	Pale yellow fine granules in a few cells +	No granules. Dirty brown colouration of a few histiocytes ?	No orange, yellow or brown colouration of histiocytes —
Lepromatous ...	21	10	9	0	2
Possibly lepromatous	1	0	0	1	0
Borderline ...	2	0	1	1	0
Tuberculoid reacting	7	0	0	0	7
Tuberculoid quiescent	13	0	0	3	10
Maculoanæsthetic	2	0	0	0	2
Healthy skin ...	2	0	0	0	2

It will be seen from Table I that definite intracellular lipoids were found in 19 of the 21 lepromatous cases. They were absent in all the tuberculoid cases (whether reacting or quiescent) and in the two maculo-anæsthetic cases and the two specimens of healthy skin. Of the remaining three cases (borderline 2, and ?L1) lipoids were present in one borderline case. These findings confirm the previous conclusion that presence of intracellular lipoid is almost pathognomonic of the lepromatous nature of the lesion.

3. Prognostic value of the lepromin test in contacts of leprosy cases.

The lepromin test is generally considered to be of great prognostic value both in cases of leprosy and in contacts. While this is well established in relation to the cases of leprosy, the same cannot be said about its value in contacts, since no data were available in this connection. The question has recently been studied in a population which was lepromin tested several years ago. In a rural area in Bankura district a total of 803 healthy persons had been lepromin tested in two different experiments, 15 and 20 years ago, respectively. Recently, this area was visited, and the tested persons re-examined. Out of 803 persons, 123 were not available for examination having left or died. The examination of the remaining 680 persons revealed that 39 (5·7 per cent) of them were now suffering from leprosy. Of the 39 cases, 15 were of the lepromatous and 24 of the non-lepromatous types. A correlation of the results of the lepromin test (15 to 20 years ago) and the subsequent development of the disease and type of the disease as revealed by the present examination has given interesting results, which are summarized in Table II:—

Table II.

Results of the initial lepromin test	Number of persons	Number and per cent in which leprosy developed :		
		L	N	Total
Negative ...	156	15 (9·6 per cent)	7 (4·4 per cent)	22 (14·0 per cent)
Positive				
Weak+	163	...	9	9(5·5 per cent)
Moderate+	125	...	3	3(2·4 per cent)
Strong+	236	...	5	5(2·1 per cent)
	524	...	17	17 (3·2 per cent)
Total ...	680	15 (2·2 per cent)	24 (3·5 per cent)	39 (5·7 per cent)

L = Lepromatous. N = Non-lepromatous.

It will be noticed that the incidence of the disease in the lepromin negative group has been much higher (14 per cent) than in the lepromin positive group (3·2 per cent). Moreover, in the lepromin negative group 2/3rds of the cases have been of the lepromatous type, whereas in the lepromin positive group there has not been a single case of this type.

In one of the experiments (the first one 20 years ago) an attempt has been made to increase the reaction to lepromin by repeated testing and for this purpose a total of three tests had been done in the course of one year. This was done in 109 lepromin negative individuals. In 93 of these the reaction was found to have become positive while it continued to be negative in the remaining 16. It is difficult to say as to what extent this conversion in the reaction from negative to positive was the result of repeated testing; however, for a strict assessment of the value of a spontaneous positive lepromin reaction, these 93 individuals should be excluded from the analysis. A correlation of the results of the lepromin test in the remaining 587 persons with subsequent development of leprosy and type of the disease is shown in Table III:—

TABLE III.

Results of the lepromin test	Number of persons	NUMBER AND IN WHICH LEPROSY DEVELOPED:		
		L	N	Total
Negative ...	63	14 (22·7 per cent)	3 (4·7 per cent)	17 (27·0 per cent)
Positive ...	524	...	17 (3·2 per cent)	17 (3·2 per cent)

The above correlation brings out even in a more clear-cut manner the fact that compared to the positive reactors, the negative reactors are not only more likely to get the disease, but also more likely to get the serious (lepromatous) form of the disease.

Of the 63 lepromin negative persons included in Table III, there were 16 in whom repeated testing was made, but the reaction continued to be negative. A study of these 16 persons brings out the interesting information that as many as 10 of them later developed the disease, and in 8 of the cases the disease was of the lepromaous type. This would tend to show that the prognosis with regard to the development of the disease and that too of the serious type is worst in persons in whom a negative lepromin reaction persists.

4. THERAPEUTIC STUDIES.

(*i*) *Dihydno-DDS.*—Last year were reported the pharmacological studies with this drug, and to some extent its therapeutic effects. As the preliminary results were encouraging, the therapeutic investigation with the drug was continued and the results are reported below:—

Number and type of patients.—A total of 35 patients was included in the investigation, 27 of the lepromatous and 8 of the non-lepromatous type. While all the eight non-lepromatous cases continued treatment for adequate periods, six of the lepromatous cases discontinued within ten weeks and another three within twenty weeks; in view of the very much longer duration of treatment in the remaining cases, all these nine cases have been excluded from the analysis. The findings reported herein are, therefore, based on 26 (18 lepromatous and 8 non-lepromatous).

Of the non-lepromatous cases, seven were tuberculoid, with a few lesions in two cases (T_1), and with the disease fairly extensive in five cases (T_2), two of which were bacteriologically positive showing a small number of bacilli. The remaining one non-lepromatous case was of the maculo-anæsthetic type with fairly extensive lesionsion (MA_2) and bacteriologically negative. All were lepromin positive. Only one of the cases was previously untreated, the other seven had been previously treated with other drugs, without any or permanent benefit.

Of the 18 lepromatous cases the disease was fairly extensive in 16 (L_2), and very extensive (L_3) in two cases. All the cases were bacteriologically positive and lepromin negative. Three of these were previously untreated, and the remaining 15 had been previously treated with other drugs without any or permanent benefit.

Duration of treatment.—Leaving out the nine lepromatous cases treated for short periods (as stated above) the duration of treatment in the remaining 26 cases varied from 30 to 106 weeks—up to 50 weeks in 7, between 60 and 100 weeks in 11, and beyond 100 weeks in 8 cases. The average duration of the treatment was 72 weeks in non-lepromatous cases, and 70 weeks in the lepromatous cases.

Mode of administration and dosage.—Parenteral administration of the suspension of the drug in hydnocarpus oil or its ethyl ester resulted in many cases in the production of an abscess at the site of injection. When given by mouth, the drug was, however, well tolerated and there were no toxic symptoms; in most of the cases there was no fall in the R.B.C. and hæmoglobin values, in 4 of 26 cases there was a slight fall in the beginning, but later it was restored completely in two and to a considerable extent in the other two. All the four cases in which slight fall in R.B.C. and hæmoglobin was seen, were of the lepromatous type. There was no detectable sign of liver damage as judged by the results of various tests (Vanden Bergh, thymol-turbidity, Schlesinger's tests).

Results of treatment.—All the cases showed improvement which could be graded as marked (complete or almost complete subsidence) in 13, moderate (considerable subsidence) in 9, and slight (definite but slight subsidence) in 4 cases. The therapeutic effect in the lepromatous and non-lepromatous cases is sumarised below:—

Lepromatous.—Of the 18 lepromatous cases there has been complete or almost complete clinical subsidence in eight cases, three of them having become bacteriologically negative, and in the remaining five there has been a reduction in the number of leprosy bacilli, the final Bacteriological Index (B.I.) being 0·5 . In six cases there has been considerable clinical and bacteriological improvement, the final B.I. varying from 0·6 to 1·8. In the remaining four cases there has been definite, though slight improvement, both clinical and bacteriological, the final B.I. varying from 1·6 to 2·5, the B. I. before treatment having been from 3·0 to 3·5.

Non-lepromatous.—All the eight cases showed marked clinical improvement. In the seven tuberculoid cases this was evidenced by the subsidence of erythema and thickening of the patches, and disappearance within 20 weeks of treatment of the leprosy bacilli in the two cases that were bacteriologically positive in the beginning. In the single maculo-anæsthetic case, the hypopigmented patch faded and the skin became almost normal. In six of the eight non-lepromatous cases there was partial restoration of sensation, and no change in this direction in the other two.

The above results would tend to show that hydnosulfone has produced satisfactory clinical and bacteriological improvement in most of the cases treated for adequate periods. The improvement became evident generally by the end of the fourth month of treatment. It is interesting to note that most of the cases included in the present series had been previously treated without any or any lasting effect.

It can be concluded that 'hydnosulfone' is of definite value in the treatment of leprosy, particularly in cases which cannot tolerate and/or do not make satisfactory progress with some other drugs, such as DDS, INH and thiosemicarbazone.

(*ii*) *Dihydroxy-DDS.*—Our preliminary investigations showed that in doses similar to DDS, dihydroxy-DDS was less toxic to laboratory animals and that the *in vitro* activity against culture of an acid-fast bacillus (Kedrowsky's bacillus) was of the same order as that of DDS. It was, therefore, decided to try the compound in cases of leprosy to see if it produced results better than DDS. Because of the limited supply of the drug only six cases, all of the lepromatous type, were included in the investigation.

Of the six cases, two had treatment for only thirteen weeks as they could not tolerate adequate dose due to occasional febrile reaction and showed no clinical or bacteriological improvement; treatment with this drug was, therefore, discontinued. The other four cases continued treatment for 38 to 53 weeks. The drug was given by mouth beingning with 25 mg. daily and gradually increasing to 100 mg. to 150 mg. In one of the cases the dose could not, however, be increased because of repeated reactions and persistent anæmia due to hookworm infection.

In the one case in whom the dose could not be raised beyond 25 mg. there was no clinical but slight bacteriological improvement. The other three cases made definite clinical and bacteriological improvement during the first few months of treatment. An outstanding feature of the improvement was the bursting of nodules followed by their healing. During the later months of treatment, however, the progress was not maintained and the results at the end of about a year's treatment were considered unsatisfactory.

It can be concluded that as judged by the results in the small number of cases treated for an adequate period, dihydroxy-DDS possesses no advantage over plain DDS. Although the results at the beginning gave rise to hopes of the new remedy being better than DDS, the final results did not come upto this expectation.

(*iii*) *INH in combination with DDS.*—Conflicting reports had been made by various workers regarding the value of INH in the treatment of leprosy. Results previously reported under this inquiry provided an explanation for these conflicting reports. It was found that INH was of definite value in the first eight to twelve weeks, specially in reducing the bacteriological concentration, but that on the whole it is not very effective in the treatment of leprosy, since there is usually a setback in the initial improvement after this period. It was assumed that the setback seen after initial improvement was possibly due to the development of INH resistant strains of the leprosy bacilli early in the course of treatment. On the basis of this assumption, it was considered that the use of INH in combination with some other anti-leprotic drug (sulphone or thiosemicarbazone) may prove more effective by delaying the development of drug resistance. This matter has been investigated by using INH in combination with DDS, and the results are reported below:—

Number and type of patients.—Twenty-four cases of leprosy of the lepromatous type were put on the combined treatment with INH and DDS. Of these cases, six were ' new ' and received combined treatment from the start. The remaining 18 cases had been treated for varying periods with thiosemicarbazone (nine cases), DDS or its derivatives (four cases), and an indigenous preparation (five cases). The reason for the change of treatment in these cases was absence of satisfactory improvement in some, and setback or standstill condition after initial satisfactory improvement in others.

Dose, route and interval.—Both the drugs were given by mouth daily for six days a week with rest on the seventh day. The initial dose of INH was 50 mg. daily which was quickly increased to 200 mg., whereas the initial daily dose of DDS was generally 25 mg. (or even lower in a few cases) and was slowly raised to a maximum of 100 mg.

Duration of treatment.—Of the 24 cases under review, one had treatment for only one week, and another for only six weeks, these two cases have not been included in the analysis. The treatment in the remaining 22 cases varied from 20 to 103 weeks—from 20 to 30 weeks in 9, between 50 and 90 weeks in 10, and above 90 weeks in 3 cases.

Therapeutic effects.—The results may first be considered as a whole, and then according to duration of treatment.

Results as a whole.—Clinical and bacteriological improvement has been observed in all cases under treatment. For the purpose of analysis the degree of improvement has been graded into slight, moderate, and marked. Slight improvement implies definite though slight clinical subsidence, and reduction of B.I. by less than 1; moderate improvement implies considerable clinical subsidence and reduction of B.I. about by two (being between 0·6 and 2); marked improvement implies complete clinical subsidence with B.I. down to between 0 to 0·5.

Taking all the cases together the results can be summarized as under:—

TABLE IV.

IMPROVEMENT:

Degree	Clinical	Bacteriological
Slight	7	7
Moderate ...	8	9
Marked ...	7	6

Results according to duration of treatment.—An analysis of the results according to the period of treatment gives additional information and is summarized below:—

TABLE V.

Duration of treatment in weeks	Number of cases	DEGREE OF IMPROVEMENT:		
		Slight	Moderate	Marked
20 to 30 ...	9	6	2	1
50 to 90 ...	10	1	5	4
Above 90 ...	3	...	1	2
Totals ...	22	7	8	7

It will be seen that the improvement in these cases has been progressive and that the degree of improvement is proportional to the duration of treatment. It is interesting to note that there was no setback of deterioration during the period of treatment. In all the cases there has been progressive improvement except in three cases in whom after showing considerable initial improvement the B.I. has been stationary for more than six months. On the combined treatment with INH and DDS, no setback or deterioration has been seen as was seen in the cases on INH alone.

(*iv*) *Gandhakamurutam.*—This is a crystalline powder sent through the Secretary, Indian Council of Medical Research, New Delhi, for trial. Seven cases of lepromatous type were put on the drug. The daily dose was 1·4 g. divided into two doses given as directed by the supplier of the medicine. One case stopped treatment after four weeks and the remaining six cases continued treatment for 24 to 34 weeks, average duration being 29 weeks. No clinical and bacteriological improvement could be noticed in these cases during the period of treatment as prescribed by the supplier. The treatment was, therefore, given up.

2. Leprosy inquiry under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

1. During the year under review 229 skin biopsies were obtained: 24 from maculæ in contacts, 42 from lesions in suspected 'dimorphous' cases, 55 from lesions in cases under treatment, 73 from lepromin reactions and 35 biopsies referred from outside. It has, however, been possible to study carefully only 57 of these biopsies.

2. A composite study of early lesions in leprosy, in the course of the last two years, has revealed an interesting feature. It was found that a number of cases presented clinical features that were not typical of either the tuberculoid or the lepromatous type of leprosy. In these cases large or small ill-defined macules with some sensory impairment were encountered. The lepromin reaction was either weekly positive or negative and bacteriological examination revealed small numbers of scattered acid fast bacilli or occasional clumps of such organisms. On histological examination, cellular infiltrates localized around the skin appendages were

found. The cellular reaction consisted predominantly of round cells and histiocytes and occasional epitheloid cells. Rarely, a few giant cells or finely vacuolated pre-Virchow cells were also encountered. Invasion and fraying of the nerve twigs, though observed, was inconstant. Bacilli were usually detected amongst the nerve fibres.

So far, 25 such cases, the majority from the Acworth Leprosy Home, have been studied in detail. It would apear that on the basis of clinical, immunological, bacteriological and histological studies, these cases manifest lesions partaking of the various characteristics of both tuberculoid and lepromatous types of leprosy. Thus, they constitute a clear entity a ‘ dimorphous ’ macule, whose existence has been doubted in the past by some leprosy workers.

3. Histological observations on the lepromin reaction appeared to confirm the findings reported last year. In a few cases of probable ‘ dimorphous ’ type of leprosy, the early (24 hours) lepromin reaction was seen to consist of a 4 mm. to 5 mm. zone of erythema. Histology revealed polymorphonuclears, histiocytes and eosinophils, and very few or no bacilli. The late (21 day) reaction in these cases, consisted of a 3 mm. to 4 mm. area of brownish discolouration or superficial infiltration. The cellular reaction around the neurovascular bundles, was made up of histiocytes alone, or histiocytes and epithelioid cells with a few giant cells. In one biopsy, only pre-Virchow cells were observed. A.F. bacilli were usually encountered in moderate or large numbers. Thus, even in the lepromin reaction, the dimorphous cases revealed features suggestive of both tuberculoid and lepromatous types of leprosy.

4. From the cases under treatment, only a small number of biopsies could be examined.

3. Leprosy inquiry under Dr. Paul W. Brand at the Christian Medical College, Vellore.

This report is divided into four sections. The first is an account of follow-up study on 100 patients who have had sublimis transplantation operation. Second is an account of follow-up studies on 62 patients who have had an abductor opponens replacement on the thumb. The third is an account of further studies of the pathology of nerve damage in leprosy, and fourth, a summary of further experience on operative procedures for the hands, feet and face.

1. During this year an attempt has been made to follow-up all the past operations that have been performed in this clinic under the Indian Council of Medical Research inquiry. For this purpose a letter was sent out to all the cases of hand reconstruction beginning with cases who lived within reasonable travelling distance of Vellore. The first batch of letters was sent to patients who had some transplantation for clawed fingers. There was no selection of cases to whom the letters were sent, except that patients who had come from a great distance were excluded. There was encouragement given in this letter for further advice and operation if operation should prove necessary. It is felt that this is an important factor because many of the patients who have come back have done so because they felt that further improvement can be gained.

In many letters sent out for purposes of follow-up studies the patient is not given any anticipation of further investigation or advice and, therefore, only the grateful patients will bother to return. Patients who have had failures will usually not return. In response to our letter, however, we have felt that most patients who have felt dissatisfied with their result have returned for follow-up because of the hope of further correction. Our figures, therefore, probably represent a more correct proportion of poor and good results than do many other series.

The detailed analysis of these cases has taken a considerable time, and only a few follow-up cases can be studied each week. Therefore, only the first 162 consecutive studies are analysed here. The longest follow-up period is five years and six months and the shortest two months.

Clawed fingers.—This analysis covered 100 hands, including 86 index finger, 88 long fingers, 100 ring fingers and 100 little fingers. The results have been analysed under the following headings.—

1. The ability of the patient to open his hands fully.
2. The ability of the patient to close his fist in the correct sequence of movement.
3. The completeness of closure of the fist.
4. The general appearance of the hand.
5. The patient's statement of improvement.

Finally, all these have been summated to give an over-all assessment of the operative results.

All assessments have been graded into four levels: (*i*) Excellent, (*ii*) good, (*iii*) fair, and (*iv*) poor.

The open hand.—A previously clawed finger, if it is now able to open fully without a single degree of flexion limitation, is graded ' Excellent '. If it is able to open sufficiently fully to appear reasonably straight, it is graded as ' good '—(opens actively to an angle of 150 degrees). If it will not open more than 120 degrees, it is classed as a failure and graded—' poor '. Intermediate cases are ' fair '.

Two complications have been included in this assessment. One is the over-correction of clawing which we have called ' intrinsic plus '. This means that when the patient straightens his fingers, they tend to bend backwards. This is not a servere disability, but if it is sufficiently marked, it is unsightly. It changes the assessment in the following way. If the finger bends backwards a little when the finger is extended, that changes the assessment from ' excellent ' to ' good' . If it bends backwards sufficiently to be obvious, it changes the assessment from ' excellent ' to ' fair '. Even an obvious backward bending is not regarded as a poor result, because it is very much better to have a finger which will over-extend rather than a finger which will not extend enough.

Sideways deviation.—In certain hands the result of the operation has been to allow the finger to extend fully, but there is a tendency for the index finger to deviate towards the thumb while doing so. This is not usually noticed by the patient except that he will complain that while trying to pick up rice or fluids they will trickle through his hands because his fingers cannot be held pressed together. If this radial deviation is significantly present in an otherwise ' excellent ' hand, it changes the assessment to ‘ good ’. If it is markedly present, it changes the assessment from ‘ excellent ’ to ‘ fair ’. Sometimes the tip of the finger only will fail to extend. If this flexed tip is present, it affects the ‘ open hand ’ assessment, and also it is separately tabulated in the table of ‘ complications ’.

The assessment of finger extension has shown the following results:—

TABLE I.

Open hand assessment.

Fingers	EXCELLENT:		GOOD:		FAIR:		POOR:	
	Cases	Per-centage	Cases	Per-centage	Cases	Per-centage	Cases	Per-centage
Index finger ...	23	27	44	51	17	20	2	2
Long finger ...	24	27	41	47	21	24	2	2
Ring finger ...	34	34	38	38	24	24	4	4
Little finger ...	24	24	40	40	30	30	6	6
Totals ...		112		176		98		14
Average per cent		28		44		24½		3½

TABLE II.

Complication included in ‘ open hand ’ assessment.

Fingers	Failure of tip extension per cent	Intrinsic plus, per cent	SIDEWAYS DEVIATION	
			Mild, per cent	Marked, per cent
Index (86) ...	27	3	27	15
Long (88) ...	20	13	27	2
Ring (100) ...	19	10	15	1
Little (100) ...	7	0	7	2
Total (374) ...	18	6	19	5

It will be noticed that in the overall results of 374 fingers approximately 30 per cent have an excellent result. That is, a fully open hand with no complication of any sort. Forty-four per cent have a good hand, i.e., either a fully open hand with a slight tendency to bend backwards or sideways, or a hand which opens sufficiently fully for adequate function. 24·5 per cent are fair, i.e., they show improvement over their previous clawing but do not satisfy the requirements of ‘ good ’. 3·5 per cent are poor, i.e., the hand does not open satisfactorily.

Assessment of the closing of the hand.—The following factors have been assessed. When the hand is closed into a fist, if there is any stiffness in the finger joints, it will be seen that the fingers do not fully meet in the centre of the fist. If a small knitting needle is held in the fist, it can be pulled out of the fist because of the failure of the fingers to meet. This opening in the fist constitutes a partial or complete failure of the operation according to the size of the gap.

The proximal interphalangeal joint should flex beyond 90 degrees to give an excellent result. Flexion of the terminal interphalangeal joint should be sufficient to allow the pulp of the finger to meet the base of the proximal phalanx. This will usually be an angle of about 70 degrees.

TABLE III.

Closed fist.

	Excellent %	Good %	Fair %	Poor %
Closed fist	39	34	22	5

The ' mechanism ' of closure represents the correct sequence of flexing of the different joints of the finger. In the normal hand, when grasping a large object, the metacarpo-phalangeal joints initiate the closing movement and the interphalangeal joints continue the movement after the object of the grasp has been surrounded by the finger. In the claw-hand, there is no muscle to initiate the metacarpo-phalangeal flexion. Interphalangeal joints flex first, and metacarpo-phalangeal joints are only closed when the others have completely closed. This feature of the claw-hand is a very disabling, and crippling one. The correction of the sequence of joint closure is, therefore, even more important than the ability to open the hand completely. It will be seen in Table IV that 90 per cent of cases have shown an excellent or good result in the correction of the mechanism of closing. This is higher than the percentage for the completely open hand, and it indicates that the operation used for it has fairly satisfactory results.

TABLE IV.

' Mechanism of closing '.

	Excellent	Good	Fair	Poor
Mechanism of closing ...	51	39	7	3

The patient's unprompted statement about his satisfaction with the operation is also tabulated. In spite of warning to the contrary many patients hope that sensation will return following operation. As it does not do so, they remain only partly satisfied.

TABLE V.

Final finger assessment.

	Excellent	Good	Fair	Poor
Appearance	12	47	37	4
Function	27	44	25	4
Patient's statement ...	15	48	33	4
Overall assessment ...	22	48	25	4

Final assessment of the operative result.—The assessor's general impression of the function of the finger including the mechanism of opening, mechanism of closing, the completeness of grasp and the appearance of the fingers. It will be seen that about five per cent of all fingers are classed as failures for one or other reason. Of the remainder about 25 per cent are improved but do not satisfy the surgeon enough to be classed as good. About 70 per cent of all the fingers are good or excellent.

Approximately, 30 per cent of the patients have replied to the letter and have returned for follow-up assessment. The patients who have not returned for assessment have all had pre and post-operative assessments done at the time of their discharge and recorded in their notes. They have also had pre- and post-operative photographs taken of their hands in six standard positions, the open hand, the closed hand, the pinch, etc. By comparing the early post-operative results of those who returned for later assessment, it can be seen that those who returned are a fairly representative cross section of the good and bad results.

An assessment is now being carried out of individual complications of the operations and their association with the pre-operative condition of the patient and the type of the operation that had been performed. This assessment is giving useful information about the indications and limitations of certain operative procedures and has pointed out the need for certain revisions and the developments of new procedures in different types of deformity.

Section II.

The thumb.—Sixty-two thumbs have so far come back for follow-up study. The length of follow-up covers approximately the same period as that for the other fingers. In many cases the thumbs are from the same hands which have had claw finger corrections. The success of operations on a paralysed thumb is judged by the following criteria:—

(*i*) The ability of the patient to bring the thumb forward in front of his fingers ready to oppose them in the action of pinch. In this position the thumb stands erect at right angles to the palm with its pulp facing the fingers. We have assessed the ability of the thumb to stand up in this way (abduction) and graded it ' full ', 'medium ' and ' poor '.

(*ii*) We have assessed separately the ability of the thumb to rotate and face the fingers pulp to pulp (opposition).

(*iii*) The next assessment is that of the action of pinching, that is, of bringing the thumb and fingers together in position to grasp a pencil or other object. This assessment is difficult because a certain clumsiness frequently remains to the patient even when his muscular action is perfect. The clumsiness is due to the fact that the fingers and thumb cannot feel each other and, therefore, are liable to slip sideways when pressing against each other. With practice the patient may learn to overcome this instability, but as we are unable to restore sensation to the thumb, some uncertainty of pinch usually remains. The grading of pinch is according to how the thumb meets the fingers. ‘pulp’ is excellent. ‘tip’ is not so good. ‘nail’ and ‘side’ are poor. It will be noticed that 79 per cent achieve good or excellent abduction and that 89 per cent have a satisfactory apposition.

TABLE VI.

Assessment of operations on 62 thumbs.

(*a*) *Abduction of thumb, 62 cases.*

FULL:		MEDIUM:		POOR:	
Cases	Per cent	Cases	Per cent	Cases	Per cent
49	79	9	15	4	6

(*b*) *Opposition of thumb.*

EXCELLENT:		GOOD:		FAIR:		POOR:	
Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent
18	30	37	59	3	5	4	6

(*c*) *Pinch.*

PULP (EXCELLENT):		TIP (GOOD):		NAIL (POOR):		SIDE (POOR):	
Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent
33	54	17	27	8	13	4	6

(*d*) *Patient's statement.*

EXCELLENT:		GOOD:		FAIR:		POOR:	
Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent
8	13	34	55	15	24	5	8

TABLE VI—(*Concld.*)

(*e*) *Overall assessment.*

EXCELLENT:		GOOD:		FAIR:		POOR:	
Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent
15	25	34	55	9	15	4	6

The time factor.—Both the thumb and finger results have also been analysed according to the length of time since operation. This has been done to answer the question which some people have about whether good results may be followed by recurrence of the deformity. It will be seen that most recent results in both the thumb and the fingers are slightly better than the results of the longer-follow-up period. The significance of this difference is difficult to analyse. As a result of changing operative techniques and increased experience in the selection of the right type of case for operation, we are undoubtedly getting much better results now than we were two, three or four years ago. These better operations should be and are reflected in better follow-up result, and this factor is difficult to distinguish from a possible factor of deterioration of originally good result as the years pass.

We will try to separate these two factors by a more careful comparison of the old follow-up study with the original operative result. We are already in a position to say that the good results from the earlier years have definitely maintained their improvement. The poor results in the earlier years have in a few cases shown an increase of deformity. The deformities that have appeared in subsequent years are not usually the original deformity but some such complications as ‘ intrinsic plus ’, i.e., the tendency to over-correction. In spite of this, it is satisfactory to observe that of the six cases which have been re-assessed after four or five years since operation, four have excellent ’ and ‘ good ’ results and only one has a ‘ poor ’ result. In the thumb assessment, of the eleven cases that have been assessed after more than three years seven have an ‘ excellent ’ or ‘ good ’ result still and only one has a ‘ poor ’ result. It is, therefore, clear that there is no significant deterioration with time.

TABLE VII.

Finger operation assessed according to length of follow-up.

Period	Number	Excellent	Good	Fair	Poor
Under 1 year ...	35	16	15	5	1
1 to 2 years ...	39	5	20	12	2
2 to 3 years ...	5	0	2	3	0
3 to 4 years ...	15	0	8	6	1
Over 4 years ...	6	1	3	1	1

TABLE VIII.

Thumb operation assessed according to length of follow-up.

Follow-up period	EXCELLENT		GOOD		FAIR		POOR	
	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent
Under 1 year : 23 cases	5	22	16	70	2	8	0	0
1 to 2 years : 23 cases	7	30	10	43	3	13	3	13
2 to 3 years : 4 cases	1	20	3	60	1	20	0	0
Over 3 years : 11 cases	2	18	5	46	3	27	1	9

Further studies on the pathology of nerve damage in leprosy.—The significance of localized swelling in the main nerves of the arm and leg is under investigation. It seems that the older leprosy workers assumed that the paralysis of the hand and foot originated in these swellings. There was some speculation as to why these swellings occurred in the positions that they did, but it was generally felt that whatever their cause, they were the primary sites of nerve damage. In 1895, however, Dehio and Woit published their own observations together with the careful studies and dissections of Gerlach, and came to the conclusion that all neurological involvement in leprosy started in the sensory nerves of the skin. The motor nerves became paralysed only when they joined a sensory nerve which had been infiltrated from the periphery. This theory was based on careful studies and has proved acceptable up to the present time. Even now, we must agree that it represents the facts in a large number of cases. It is easiest to demonstrate in the major tuberculoid form of leprosy, where a thickened cutaneous nerve may be traced from an anæsthetic skin patch proximally until it meets a mixed nerve. The mixed nerve will be thickened from the point at which it is joined by the involved sensory nerve, and motor branches given off below this area may be paralysed although not themselves thickened or infiltrated.

This theory does not explain, however, why very many nerves in leprosy are involved by the disease in the sense that they may contain numerous bacilli and may contain a cellular reaction, but yet they are not paralysed. In most cases of lepromatous leprosy a very large number of cutaneous nerves will be found full of bacilli and yet there will only be a minimal anæsthesia. Also mixed nerves of the limbs—ulnar, median and radial—may all show evidence of infection and yet may show neither severe sensory change nor even the slightest motor involvement.

This theory also fails to explain why in some cases the nerve in the swollen area may show severe and gross pathological change, while the nerve distal to it, even the sensory cutaneous branches may show very little change, except for the disappearance of a number of neurons which have been compressed to extinction in the swollen area above.

It has seemed to us in studying the paralysis of leprosy that we have to recognize three types of reaction of the nerve to the lepra bacillus. First, there is the overwhelming tissue response to a very small number of bacilli that occurs typically in the major tuberculoid type of leprosy. It seems as if any nerve which gets infected or involved by the disease in such cases is likely to be paralysed. It is probably not a simple direct action of the bacillus on the axis cylinder, but is due mainly to the severe tissue response in the strongly lepromin positive type of case.

At the other extreme, we have the early lepromatous type of case which shows almost no immune response to the bacilli. Numerous bacilli seem to be able to grow and multiply within the nerve causing only a minimal cellular reaction and minimal interference with nerve conduction.

The third type of nerve reaction is common to a variety of types of leprosy. It occurs in lepromatous cases when the later paralytic stage is reached. It occurs in tuberculoid leropsy when it is less strongly reactive than major tuberculoid, and it occurs in intermediate forms. In these types of cases, there may be a few, or many bacilli scattered throughout the nerve and there may be areas of severe cellular reaction and areas which are almost free of reaction. It is in these cases that the anatomical situation of the nerve may be critical in determining both the severity of the nerve swelling and the presence or absence of paralysis.

The nature and mode of action of the factors that make for severe reaction in one part of the nerve and minimal reaction in another part have yet to be determined. At this time we are working actively at this problem.

It has seemed to us important, however, to demonstrate that the Gerlach theory of ascending nerve damage is incomplete and perhaps even untrue when applied to this third type of case. If the damage were purely an ascending sensory one in all cases, then there would be no need to postulate local anatomical factors at the sites of election for nerve swellings and, there would be no special impetus to find out the nature and overcome the effect of these factors.

ARGUMENT.

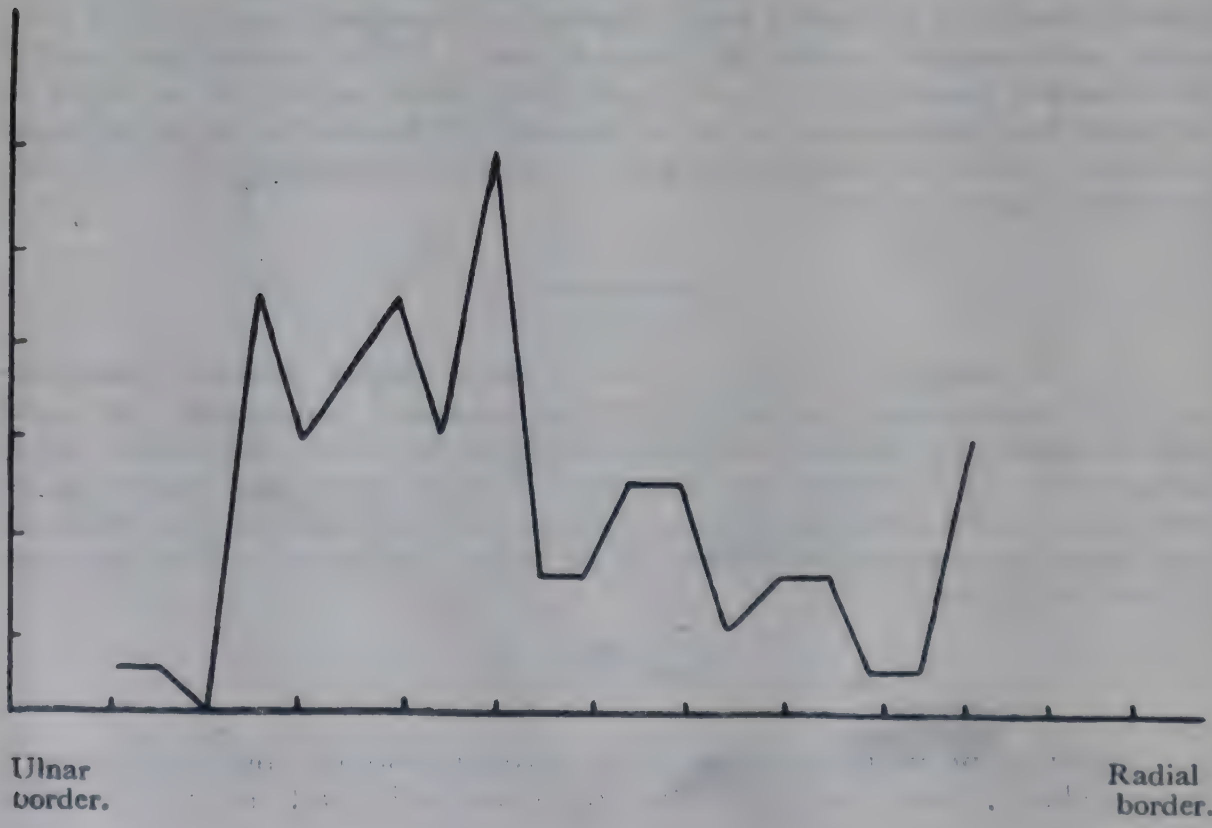
If the peripheral sensory damage were always primary, then the pattern of paralysis on the hand would be somewhat haphazard. It would depend upon the shape of a local anæsthetic patch or upon some other such factor. If, however, the anæsthesia on the hand may sometimes be secondary to the swellings in the forearm, then the pattern of anæsthesia will follow the sensory distribution of the main arm nerves, rather than any haphazard pattern.

THE STUDY.

During the past five years, every patient who has presented himself at the Hand Clinic for advice has been assessed, and notes have been kept of that assessment. Part of each assessment has been the mapping out of his anæsthesia to light touch and to pin prick, and marking this anæsthesia on a printed diagram of the hand. These diagrams of anæsthesia have been filled in by different doctors and technicians. At the time that they were filled in, it was not intended to use these diagrams for the purposes of basic nerve studies, but only as a record of the progress of surgical case. The analysis of these diagrams, therefore, should give a completely unbiased result. By far the greater number of these patients had complete anæsthesia of the hand and, therefore, were of no interest in this study. Of those who had partial anæsthesia of the hand it was found that every single one had anæsthesia of the ulnar border. This ulnar border anæsthesia in some cases was a narrow band in other cases seemed to spread across approximately to the junction between the territory of the ulnar nerve and of the median nerve. In other cases, it spread most of the way across the palm.

Owing to the fact that the division between the ulnar and median territory in the palm is not at exactly the same level in every person, and owing to the fact that different assessors made these diagrams, it was a difficult task to think of any way of demonstrating that the border between the ulnar and median territories of the hand was respected by the paralysing factor in the nerves. Finally, the following simple method was used.

In every case of partial anæsthesia of the hand the standard printed diagram was measured to determine the width of the anæsthetic band on the ulnar side of the hand, measuring from the ulnar border towards the thumb. The measurement was made in millimetres and the widths were grouped together, so many cases 5 mms. wide, so many cases 6 mms. wide, etc. A graph was then plotted showing the number of cases that had different widths of anæsthesia measured from the ulnar border. The resulting Graph is reproduced below:—



It will be seen that there are a few cases in which there is a very narrow band of anæsthesia, involving only part of the ulnar nerve and none of the median territory. These cases do not affect the argument one way or the other. The striking feature of the Graph, however, is that there is a high spike or series of spikes in the area 5 mm. to 10 mm. On the Graph these figures represent an area on the palm which compares almost exactly with the average extent of the ulnar nerve territory as shown in anatomy text-books, and checked by ourselves by anæsthetizing the ulnar nerve with novocaine. Once this critical point has been passed, the number of cases that show anæsthesia involving ulnar and part of median territory of the palm drops suddenly to a small figure.

These figures are difficult to analyse statistically, because of the number of variable factors involved, but a glance of the Graph seems to demonstrate very effectively the point that we are trying to prove. It is simply that a very significant proportion of the cases that have partial anæsthesia of the hand show the limitation of the anæsthesia at or very near the junction of the ulnar and median territories of the palm.

If the anæsthesia were truly peripheral in origin, this would be a most astonishing coincidence.

We suggest, therefore, that in many cases, the actual nerve damage and destruction (as opposed to infection by bacilli or infiltration by cells) is primary in the nerve trunks and nerve bundles of the arm and leg, rather than being primary in the nerve endings.

It must be made clear that we are discussing arm and leg only, not the anæsthesia of patches on the body.

Since in the past two years our reports have emphasized on new operations, we will not report in detail on new procedures in hand and foot surgery. It is enough to report that further experience is being gained and new procedures are being added and that old procedures are under constant review.

We should maintain, however, that further experience is being gained on the surgery of the nose. We have been using composite cartilage graft taken from the costal cartilages and jointed to make an 'L' shaped angle after being boiled for 5 minutes. These are very simple and effective. With their use, it seems necessary only to separate the skin of the nose widely from the deeper structures and then to slip in a pre-formed nasal skeleton which reaches from the frontal bone to the tip of the nose and then turns down through the columela and takes a firm seating in the maxillary bone. After further experience with this method it will be more fully reported in the surgical literature and in these reports.

4. Leprosy inquiry under Dr. S. D. Desai at the Acworth Leprosy Home, Bombay.

I. (*a*) *Examination of new contacts attending during* 1954-55.

Table I gives the results of the clinical and bacteriological examination of new contacts of both neural and lepromatous patients.

TABLE I.

Total number of contacts examined ...			245
(i) Number of positive contacts (infected contacts without lesions): ...	Adults ...	39	
	Children ...	26	
	Total ...		65 (26·53 per cent)
Sixty-two were positive and three (children) negative to lepromin.			
(ii) Number of contacts with primary or basic lesions:	Adults ...	1	
	Children ...	11	
	Total ...		12 (4·89 per cent)
All were positive to lepromin.			
(iii) Number of contacts with persistent lesions-early neural:	Adults ...	3	
	Children ...	*Nil*	
	Total ...		3 (1·22 per cent)
All were positive to lepromin.			
(iv) Number of contacts with neural leprosy (tuberculoid or anæsthetic macules)	Adults ...	17	
	Children	45	
	Total ...		62 (25·30 per cent)
(v) Number of contacts with lepromatous leprosy:			*Nil*
(vi) Number of negative contacts (uninfected contacts) ...	Adults ...	50	
	Children ...	53	
	Total ...		103 (42·04 per cent)
Thirty-nine positive and 64 were negative to lepromin.			

I (*b*) *Examination and follow-up of old contacts of previous years.*

One hundred and fifty-two contacts examined during the previous years were followed up during the year. The condition noted from time to time in these 152 contacts is grouped and described as follows:—

(*i*) Forty-eight (17 adults and 31 children) negative contacts (uninfected) were observed during a period of four months to six years. Of these, 20 (eight adults and twelve children) became positive contacts (infected, without lesions).

Thirteen (2 adults and 11 children) developed primary lesions. Infected stage was not observed.

Two (children) developed neural leprosy (one anæsthetic and one tuberculoid macule). Intermediate stages were not observed.

Thirteen (seven adults and six children) remained uninfected.

Of the 20 positive who got infected:

Six (one adult and five children) developed primary lesions.

In one of the six the primary lesion vanished later.

One (child) developed a tuberculoid macule. The earlier stages were not observed.

One (adult) developed persistent lesion-early neural after passing through the primary lesion stage.

Three (two adults and one child) became negative for bacilli though the lepromin was still positive.

Nine (four adults and five children) remained in the infected stage.

Of the 13 who developed primary lesions, the infected stage not having been observed :

One (child) developed a tuberculoid macule after passing through the persistent lesion-early neural stage.

Eleven (two adults and nine children) remained static.

In one (child) the primary lesion vanished.

(*ii*) Fifty-two (31 adults and 21 children) positive contacts (infected, without lesions) were observed during a period of four months to six years. Of these:

Sixteen (5 adults and 11 children) developed primary lesions.

One (child) developed persistent lesions-early neural. The primary lesion stage was not observed.

One (adult) developed an anæsthetic macule. The primary lesion and the persistent lesion-early neural stages were not observed.

Eight (adults) became negative for bacilli though the lepromin was still positive.

Twenty-six (17 adults and 9 children) remained in the infected stage.

Of the 16, who developed primary lesions:

One (adult) developed persistent lesion-early neural.

One (child) developed a tuberculoid macule after passing through the persistent lesion-early neural stage.

Two (children) developed tuberculoid macules. The persistent lesion-early neural stage was not observed.

In two (one adult and one child), the primary lesions vanished.

In ten (three adults and seven children), the primary lesions remained static.

(*iii*) Fifty (11 adults and 39 children) contacts with primary lesions were observed during a period of four months to six years.

Of these:

One (adult) developed persistent lesion-early neural.

Two (children) developed tuberculoid macules after passing through the persistent lesion-early neural stage.

Six (one adult and five children) developed tuberculoid and anæsthetic macules. The persistent lesion-early neural stage was not observed.

In ten (three adults and seven children), the primary lesions vanished. Of these, two adults and three children became negative for bacilli in addition, though the lepromin was positive.

In 31 (6 adults and 25 children), the primary lesions remained static.

(*iv*) Two (children) with persistent lesion-early neural were observed during a period of nine months to one year and five months.

Of these, one developed a tuberculoid macule in one year and five months and the other remained static.

Note.—(1) The average period of time during which the uninfected contacts got infected was two years and one month in adults and one year and three months in children.

(2) Primary lesions developed in infected contacts in an average period of one year and one month in adults and one year and eight months in children.

(3) Primary lesions developed into clinically distinguishable lesions (persistent lesions-early neural, anæsthetic or tuberculoid macules) in an average period of one year in adults and one year and four months in children.

II. *Results of examination for acid-fast bacilli in suspicious cases directed to the Clinic of the Acworth Leprosy Home, Bombay.*—Four hundred and thirty-seven persons were examined during the year 1954-55 for acid-fast bacilli. None of them was found to be suffering from leprosy. The results of the bacteriological examination by the chloroform extraction method (of Figueredo and Desai), showed negative results in 403 persons. Eleven of these who could be re-examined were again negative for acid-fast bacilli. One of these who could be re-examined a third time was negative again.

Thirty-four persons showed a few acid-fast bacilli, four to ten in number, on the first examination. In only 19 of these could a repeated examination be done. Of these, 17 gave negative results and two showed bacilli. Only one of these two showed a few acid-fast bacilli on the third examination. It was not possible to determine whether these persons were contacts of leprosy cases.

The percentage of positives on the first examination is, therefore, 7·78 (34 out of 437) and the percentage of positives on repeated examinations is 0·46 (2 out of 437).

The percentages (7·78 and 0·46) when compared to the percentage of contacts showing acid-fast bacilli (26·53 per cent) reveal a very significant variation and support the opinion that the acid-fast bacilli found in contacts are *M. lepræ*.

III. *Study of the prophylactic value of DDS in small doses.*

Table II gives the number of negative contacts (uninfected persons) treated with DDS in varying doses and of those not treated with DDS and their status after a period of 5 to 16 months.

TABLE II.

	Number of contacts	Type of infector	Period of treatment or observation in months	Dose of DDS in mg. twice a week	Results
Treated group	2	Neural	12—16	25	No change
Adults ...	4	3 lepromatous 1 neural	6—10	1 on 50 2 on 25 1 on 10	No change (N-infector) No change (L-infectors) No change (L-infector)
Control group	2	1 lepromatous 1 neural	12—16	...	1 No change (N-infector) 1 got infected (L-infector)
Adults ...	2	Neural	5—10	...	No change
Treated group	2	Neural	12	1 on 25 1 on 10	No change No change
Children	2	1 lepromatous 1 neural	5—6	10	No change
Control group	5	Neural	12—16	...	Two—no change. Two got infected. One got infected and developed primary lesions.
Children	10	5 lepromatous 5 neural	5—10	...	Two no change (L-infectors). Five got infected (2 L-infectors & 3 N-infectors). Three got infected and developed primary lesions (1 L-infector and 2 N-infectors)

IV. *Study of the effect of small doses of DDS in positive contacts (infected persons without lesions)*.—Table III gives the number of positive contacts treated with DDS in varying doses and of those not treated with DDS and their status after a period of 5 to 21 months.

	Number of contacts	Type of infector	Period of treatment or observation in months	Dose of DDS in mg. twice a week	Results
Treated group	10	2 lepromatous	11—21	4 on 50	Three became negative (N-infectors) One—no change (L-infector)
		8 neural		6 on 25	All became negative (1 L-infector and 5 N-infectors)
Adults	10	3 lepromatous 7 neural	5—10	2 on 50 8 on 25	No change (L-infectors). Seven—no change (1 L-infector and 6 N-infectors). One became negative (L-infector).
Control group	5	4 lepromatous	11—21	…	Three—no change (2 L-infectors and 1 N-infector).
		1 neural			One developed primary lesion (L-infector). One developed anæsthetic macule (L-infector)
Adults	2	1 lepromatous 1 neural	6—10	…	No change
Treated group	3	Neural	21	25	One—no change Two became negative
Children	1	Lepromatous	6	10	No change
Control group	9	6 lepromatous	11—12	…	Three—no change (2 L-infectors and 1 N-infector).
		3 neural			Three developed primary lesions (2 L-infectors and 1 N-infector). Three developed tuberculoid macules (2 L-infectors and 1 N-infector).
Children	2	Lepromatous	6—10	…	One—no change One developed primary lesions.

V. *Treatment with DDS combined with iso-nicotinic acid hydrazide.*—Seven cases (six lepromatous and one borderline) were treated for 10 to 21 months with DDS combined with iso-nicotinic acid hydrazide. The dose was, DDS, 100 mg. and I.N.H., 100 mg., daily for six days of the week. Two lepromatous and the borderline cases were rendered negative. Two showed marked bacteriological improvement and in two, there was no change.

One thousand one hundred and seventy-three cases, both lepromatous and neural, have been put on this combined treatment recently.

PLAGUE.

1. Inquiry on the pullicidal value of pyrethrum under the Director, Haffkine Institute, Bombay.

This inquiry was sanctioned with a view to determine the value of a proper formulation of pyrethrum as a pullicide which would give a good immediate knock-down effect, and would also have a sufficiently long residual effect. Last year, experiments were done to get sufficient pyrethrum extract and standardize methods for extraction and evaluation of pyrethrum. The Potter's tower machine for dusting the powder formulation was also standardized. Experiments were started in the open areas in Bombay to study the residual effect of the pyrethrum formulation.

During the year, attention has been given to the following studies:—

1. Studies on the chemical deterioration of pyrethrins and the addition of stabilizers.
2. Biological study of different concentrations of pyrethrum powder formulation in the laboratory with and without the boosters.
3. Field trials.

Chemical work.

A method was developed for large scale extraction of pyrethrum flowers with petroleum ether, (B.P. 40°C. to 60°C.), and 69 lb. 12 oz. of pyrethrum flowers were extracted yielding 1,346·26 g. of pyrethrum extract. The pyrethrin contents of this extract varied from 17·05 per cent to 30·37 per cent on the basis of the weight of the extract, and from 0·68 per cent to 1·19 per cent on the basis of the weight of the powder. The cost of the extract worked out to be Rs. 84–9–6 per lb. This extract was later compouned with China clay. The cost of the finished product for dusting as was used in the field trials came to be Rs. 0–14–0 per lb. The cost can be considerably brought down by:—

(*i*) The use of flowers of high pyrethrin content,

(*ii*) Selecting a more cheaper and suitable solvent, available in bulk packings,

(*iii*) Greatly, modifying and improving the technique of extraction to ensure lesser and lesser losses of the solvent during extraction, and more and more recovery of the solvent after extraction.

Experiments were carried out to study the deterioration of pyrethrum-extract when stored in tubes in dark and in light. Two stabilizers, hydroquinone and resorcinol were tried to see if this reduction be prevented. The results obtained with hydroquinone are given in Table I. The figures are an average of three readings. It will be noticed that hydroquinone does, to some extent, prevent this deterioration. Regarding resorcinol, it was difficult to do the estimation of pyrethrin II. But biological tests did not show that the powder with stabilizers had any better effect on the knock-down than the deteriorated pyrethrins. The deteriorated pyrethrins as a result of 90 days storage were then continued to be compounded and observed for a further period of 90 days for biological tests with and without the addition of a booster (piperonyl butoxide). The results are given in

Table II. It will be seen that even deteriorated pyrethrum by itself gave 60 per cent knock-down up to 75 days in dark and up to 30 days in the light. The addition of piperonyl butoxide to it continued to give 60 per cent knock-down up to 90 days in dark and 45 days in light, thus showing that piperonyl butoxide, a booster, did enhance the knock-down property of deteriorated pyrethrins to a certain extent.

TABLE I.

Chemical deterioration of pyrethrum and the effect of a stabilizer.

(The figures represent the percentage on the weight of pyrethrum extract. The figures are an average of three readings.)

Days	PURE PYRETHRUM EXTRACT		PYRETHRUM EXTRACT *PLUS* HYDROQUINONE	
	Dark	Light	Dark	Light
Immediate	16·27	16·27	16·27	16·27
15	15·92	14·95	16·17	15·34
30	15·51	13·82	15·90	14·99
45	15·12	12·77	15·80	13·86
60	14·72	11·87	15·61	13·72
75	14·52	10·46	15·04	12·41
90	13·90	8·51	14·80	12·10
192	12·42	5·80	...	...

Sesame-oil, castor-oil, & diesel oils were tried as boosters, but the estimation of pyrethrins in mixture with oils has not been successful. There is some evidence to show that sesamin and sesamolin, two active principles of sesame-oil help to boost pyrethrins in the same way as piperonyl butoxide. The other ingredients of sesame-oil cause a stickiness in the powder formulation, the use of pure sesamin and sesamolin only, would be valuable. Attempts are being made to isolate sesamin and sesamolin from sesame-oil. Sesame-oil by itself is shown to give a boosting effect, but the oily material interferes with the proper estimation and compounding. If sesamin and sesamolin could be isolated and used, then it would be possible to have a substitute for piperonyl butoxide because sesame oil is of indigenous origin. Piperonyl butoxide is not readily available in India and has to be imported.

BIOLOGICAL STUDIES.

The pyrethrum extract was compounded with China clay in 13 different concentrations and dusted in standardized doses from a Potter's tower in petry-dishes. One set of the dishes was kept in the dark and the other continuously exposed to light on the terrace. Three replications of each set were tested after storage for 15, 30, 45 and 60 days with a random sample of *X. cheopis* reared in the laboratory.

TABLE II.

Biological tests of deteriorated extract after 90 *days of storage with and without a booster* (*piperonyl butoxide*).

(Time of contact = 15 minutes. Number of fleas in each case = 20. (24 hours observation). Fresh powder was compounded from the deteriorated extract on the day of trial. Dose = 1 mg. of the active ingredient per sq. ft.)

Number of days tested after:	PYRETHRUM EXTRACT						PYRETHRUM EXTRACT + PIPERONYL BUTOXIDE						CONTROL (CHINA CLAY)					
	Dark			Light			Dark			Light			Dark			Light		
	D	S	L	D	S	L	D	S	L	D	S	L	D	S	L	D	S	L
90	20	...	...	17	3	...	19	1	...	18	2	...	...	3	17	...	4	16
90 + 15	20	...	...	7	13	...	18	2	...	17	3	...	...	4	16	...	2	18
90 + 30	18	2	...	6	14	...	20	...	...	19	...	...	2	...	18	3	...	17
90 + 45	18	2	...	10	10	...	20	...	...	15	5	...	...	...	20	...	...	20
90 + 60	13	7	...	2	18	...	17	3	...	6	14	...	...	...	20	...	1	19
90 + 75	12	8	...	5	15	...	13	7	...	5	15	...	...	...	20	...	...	20
90 + 90	8	12	...	1	8	11	13	7	...	4	16	...	...	...	20	...	...	20
90 + 105	8	...	12	1	...	19	...	...	...	...	...	...	...	...	...	...	...	...

D=Dead; S=Struggling; L=Alive.

Among the 13 concentrations tried, following were significant: 1 mg., 3 mg., 0·5 mg., 0·25 mgm. and 0·125 mg. of the active ingredient per sq. ft. Though initially the knock-down was observed after 1, 2, 3, 6 and 24 hours with the object of studying immediate and residual effect, later it was found convenient to confine these observations to 24 hours only. The results have been compared with DDT in China clay in similar concentrations.

Of the above concentration, 1 mg. per sq. ft. (i.e., powder containing 0·289 per cent pyrethrins) gave good results as shown in Table III from the point of view of dusting and knock-down property. Since pyrethrum was found to deteriorate to some extent even under ideal conditions of storage, it was desirable to see if the addition of some stabilizers and boosters gave better results. Thus, pyrethrum with a stabilizer and a booster was compounded and the residual effect observed. These powders were made of pyrethrum, prethrum *plus* piperonyl butoxide, pyrethrum *plus* hydroquinone, pyrethrum *plus* resorcinol, pyrethrum *plus* sesame-oil, pyrethrum *plus* castor and diesel oil. The results of 1 mg. per sq. ft. concentration have given us good figures. The results of these biological tests were submitted to the Director, Bureau of Economics and Statistics, Government of Bombay, and his remarks are appended below:—

'The data regarding a number of fleas affected by different powder formulations and their residual effect for 15, 30, 45, and 60 days were supplied to this office by the Haffkine Institute. These powder formulations are of the strength 1 mg. per sq. ft. and are based on the fact that the weight of the powder collected per sq. ft. is 345 mg. when 500 mg. of powder is dusted. The following conclusions are drawn from the results analysed in this office. These conclusions are based on χ^2 test at five per cent level of significance.

'(1) The immediate knock-down effects of Py.+P.B., Py.+S.O., Py.+C.O., Py.+H.Q., Py.+S.O.,+H.Q., and Py.+P.B.+Res., are better than DDT.

'(2) As far as the residual effect in dark is concerned, there is no difference among all the formulations including DDT for 15, 30 and 60 days.

'(3) There is no difference between pyrethrum and DDT as far as their residual effect for 60 days is concerned.

'(4) The residual effect of Py.+P.B. in light is more than that of DDT for 15 days but it is the same as DDT for 30, 45 and 60 days.

'(5) In light Py.+S.O. is as effective as DDT as far as their effect for 45 and 60 days are concerned.

'(6) The residual effect of Py.+P.B. is more than that of Py.+S.O. for 15 and 30 days only. However, the residual effects of these powder formulations are the same for 45 and 60 days.'

From the statistical observations it will be seen that pyrethrum as such does have a residual effect comparable to DDT in the same concentrations. The observations have been done up to 60 days. Of all the boosters, piperonyl butoxide gave better knock-down effect. Next to piperonyl butoxide was sesame-oil. The stabilizers alone do not have any enhancing effect on the knock-down, like the boosters.

TABLE III.

Biological test.

Dosage = 1 mg. per sq. ft. of active ingredient. Number of fleas in each trial = 20. 0·289 per cent pyrethrins. Time of contact = 5 minutes. Observations = 24 hours. The results are an average of three readings. First figure represents, Dead; Second, Alive; Third, Total number of fleas used.

Name of the formulation	Immediate knock-down	15 days		30 days		45 days		60 days		Control (average)
		Dark	Light	Dark	Light	Dark	Light	Dark	Light	
Py. ext. + China clay ...	20–0–20	20–0–20	11–9–20	20–0–20	15–5–20	19–1–20	17–3–20	20–0–20	20–0–20	4–16–20
DDT in China clay ...	13–7–20	20–0–20	17–3–20	19–1–20	20–0–20	19–1–20	20–0–20	20–0–20	20–0–20	2–18–20
Py. + P.B. 1:5	20–0–20	20–0–20	20–0–20	20–0–20	20–0–20	20–0–20	20–0–20	20–0–20	20–0–20	1–19–20
Py. + H.Q. 1:2	20–0–20	20–0–20	14–6–20	20–0–20	20–0–20	20–0–20	20–0–20	20–0–20	14–6–20	1–19–20
Py. + S.O. 1:5	20–0–20	20–0–20	7–13–20	19–1–20	13–7–20	20–0–20	20–0–20	20–0–20	18–2–20	0–20–20
Py. + C.O. 1:5	20–0–20	19–1–20	15–5–20	16–4–20	7–13–20	20–0–20	20–0–20	18–2–20	10–10–20	0–20–20
Py. + P.B. + Res. 1:5:2 ...	20–0–20	20–0–20	19–1–20	19–1–20	18–2–20	20–0–20	20–0–20	20–0–20	20–0–20	1–19–20
Py. + S.O. + H.Q. 1:5:2	20–0–20	20–0–20	17–3–20	20–0–20	20–0–20	15–5–20	20–0–20	20–0–20	19–1–20	2–18–20

Table IV.

Field trial in rat burrows.

Flea index up to 60 days.
Dose = 1 mg. of active ingredient per sq. ft. 0·289 per cent pyrethrins.

Name of locality and formulation dusted	Flea index				
	Immediately after treatment	15 days after treatment	30 days after treatment	45 days after treatment	60 days after treatment
Wadia Hospital, (control) ...	0·29	0·45	0·57	3·3	4·6
Spring Mills, pure pyrethrum dust	0·96	0·11	0·28	0·43	0·24
Sakrabai Hospital, (control)	0·25	1·7	1·7	0·34	1·2
Haffkine Institute pyrethrum +sesame-oil	0·92	0·088	0·42	0·65	*Nil*

Table V(*a*).

Field trial in the villages of the Madras State.

Formulation	Flea index before treatment	Flea index immediately after dusting	35 days after treatment	76 days after treatment
Control	1·04	3·8	2·74	3·4
0·289 per cent pyrethrum dust	1·9	1·3	0·56	0·37
10 per cent DDT dust ...	2·3	0·05	0·04	0

Table V(*b*).

Field trial in Satara.

Formulation	Flea index	
	Before treatment	15 days after treatment
Control	4·1	5·2
0·289 per cent pyrethrum dust with piperonyl butoxide ...	6·33	3·64
10 per cent DDT dust	12·0	4·87

FIELD TRIALS.

The pyrethrum powder with 0·289 per cent pyrethrins was tested at the rate of 1 mg. of the active ingredient per sq. ft. in the field in Bombay. As piperonyl butoxide was not readily available, sesame-oil was used as a booster. These trials were done from December, 1954 to March, 1955. Table IV shows the reduction in the flea index in the Bombay area observed up to 60 days (i.e., the period up to which laboratory trials were done). This pyrethrum powder has also been sent for field trials in Madras and the same mixed with piperonyl butoxide to Satara. It is being tried in the field in three villages in both places in comparison with DDT of the concentration in vogue for plague control measures. The observations in Madras refer to 76 days period but so far those in Satara refer for 15 days only. It will be noted from Tables V*a* and V*b* that while pyrethrum dust was of a concentration of 0·289 per cent pyrethrins, DDT was used in ten per cent concentration. Pyrethrum has given a residual effect so far up to 76 days in Madras. Further results are awaited. The field experiments in Madras are kindly being done under the supervision of Dr. Francis and in Satara under Dr. Patel of Public Health Department of Bombay State.

CONCLUSIONS.

It has been possible to show that stabilizing compounds do retard the reduction of pyrethrins, but they do not help in improving the knock-down property of reduced pyrethrins. It is boosters that enhance this effect and piperonyl butoxide has been shown to do this.

Field trials were given to pure pyrethrum, as well as pyrethrum with piperonyl butoxide and sesame-oil. The results so far available do show that even without a booster, pyrethrum has a residual effect up to 76 days observed so far. These trials are continuing. In the case of sesame-oil, though it gives a boosting effect, the oily material makes the powder slightly sticky and then it spreads sparcely. The boosting effect of sesame-oil is probably confined to two of its fractions sesamin and sesamolin. If these could be isolated and used alone, the stickiness of the oil would be eliminated. Piperonyl butoxide gives a very good boosting effect but is not readily available in India. Sesamin and sesamolin when isolated would be readily available to be used as a booster for incorporation in the pyrethrum powders.

2. Studies in enzymes of *Pasteurella pestis* under Dr. D. L. Shrivastava at the Central Drug Research Institute, Lucknow.

Since the submission of the last report, considerable work has been done on the screening of the enzymes of *Pasteurella pestis*. In addition to the strains reported upon last year, nine virulent and eight avirulent strains were obtained, from the Haffkine Institute, Bombay. These were periodically tested for their purity during the process of their routine screening.

The new strains also showed the presence of de-aminases, phosphatases, dehydrogenases and catalase, whereas aryl-sulphatase was found to be absent. Indications of differences in the de-aminase activities of virulent and avirulent strains reported earlier, have not been confirmed with these new strains. Tests were also carried on for the following enzymes:—

1. Tryptophanase.
2. Glutathione splitting enzyme.
3. Caseinase.
4. Nitratase.
5. Transaminases.

Among these, special mention may be made of the absence of tryptophanase and nitratase, whereas quite a few of the transaminase systems were found to be present. The ∝-ketoglutarate—leucine and the ∝-ketoglutarate—aspartic acid transaminase systems were distinctively noted. Preliminary investigations also indicated the presence of another system, viz., leucine pyruvate transaminase system.

Detailed work on various de-aminases using all the virulent and avirulent strains was completed about the middle of this year and a paper entitled 'Studies in the enzyme make-up of *Pasteurella pestis*. Part I. Deamination of amino acids by virulent and avirulent strains' has been communicated for publication in the *Indian Journal of Medical Research*. Important results of this investigation are as follows:—

Screening of de-aminase activity with 20 amino acids showed that *P. pestis* could appreciably deaminate only four amino acids, namely, serine, glycine, asparagine and cystein. Among these serine showed maximum deamination and cystein the minimum. It may be noted that serine had also been reported earlier (Rao, *Ind. Jour. Med. Res.*, **27,** 1940, p. 617) and confirmed by us, to have higher oxidative metabolism rate, as compared to other amino acids. The optimum pH and temperature for deamination were found to be between 7 and 7·5 and 37·5° C., respectively. It was also established that the cells grown in broth showed greater de-aminase activity than those grown on the solid medium. Anærobiosis considerably inhibited the liberation of ammonia from glycine and cystein, whereas with serine and asparagine the deamination was only very slightly affected. A comparison of the de-aminase activities of a large number of virulent and avirulent strains did not show any general correlation between virulence and deaminative processes of this organism.

A detailed study on the phosphatase activity of *P. pestis* was next taken up, using β-glycerophosphate as the substrate. It was found that the optimum pH for this reaction was at 8·0 after which the phosphatase activity showed a steep fall. At pH 5·0 dephosphorylation was much less, presumably indicating that acid phosphatases were absent in this organism. Optimum temperature was again found to be at 37·5° C. The enzymic activity fell very gradually up to a temperature of 42·5° C. and more abruptly beyond this temperature.

With a view to determine the most suitable medium in which the organisms could be grown to give maximum phosphatase activity, cells obtained from a number of media were screened and the amount of inorganic phosphorus released against the media employed is presented in Table I only for one of the cultures.

TABLE I.

Effect of medium on the alkaline phosphatase activity of *P. pestis* (*virulent* 124/*P*).

Medium	μg. of inorganic phosphorus released in two hours
Tryptic casein digest broth ...	4·0
Beef heart infusion broth	1·7
Papain digest broth	4·4
Tryptic casein digest agar	5·4
Beef-heart infusion agar	1·7
Papain digest agar	5·4

It will be noted from Table I that in contrast to the deaminase activity, the cells grown in solid medium were found to have more alkaline phosphatase than those grown in the liquid medium. The cells harvested from papain agar or casein agar were found to be uniformly more active than those from the heart infusion agar. Papain agar was, therefore, employed in all the later screening and other work. Table II presents the amount of inorganic phosphorus released in two hours by ten of the virulent and seven avirulent strains of *Pasteurella pestis* under closely comparable experimental conditions.

TABLE II.

The alkaline-phosphatase activity of certain virulent and avirulent strains of *Pasteurella pestis*.

Cultures (virulent)	μg. of inorganic phosphorus released in 2 hours	Cultures (avirulent)	μg. of inorganic phosphorus released in 2 hours
Fukeim China ...	4·8	53/H ...	5·4
Q ...	3·4	Massland ...	5·7
327/L ...	5·4	P ...	2·8
124/P ...	6·8	P.M. ...	2·8
8/P ...	5·7	Tjiwedj rough	6·4
337/L ...	6·0	Preanger ...	6·8
120/5H ...	7·5	Somadang	4·0
138/P ...	7·2		
Vimla Bai ...	4·4		
195/P ...	7·1		
53/H ...	4·4		

It will be seen from Table II that all these strains dephosphorylate β-glycerophosphate, and the amount of inorganic phosphorus released varies from 2·8 μg. to 7·5 μg. There does not, however, appear to be any general relationship between the virulence and phosphatase activity. The time reaction curves of phosphatase activity with eight strains of *P. pestis* revealed that there was a steady rise in activity up to 3·5 hours, after which a steady level was achieved. This work on alkaline phosphatase has been completed and is being written for publication.

Oxidative metabolism of virulent and avirulent strains of *P. pestis*.—Studies on the oxidative metabolism using a few of the sugars, fatty acids and amino acids have also been undertaken and the detailed studies with all the cultures are in progress.

It is expected that during the year the detailed work on the oxidative metabolism of the virulent and avirulent strains of *P. pestis* using various sugars, fatty acids and amino acids as substrates will be completed.

3. Inquiry on the mechanism of the maintenance of plague infection in rats and fleas under Dr. S. C. Seal at the All-India Institute of Hygiene & Public Health, Calcutta.

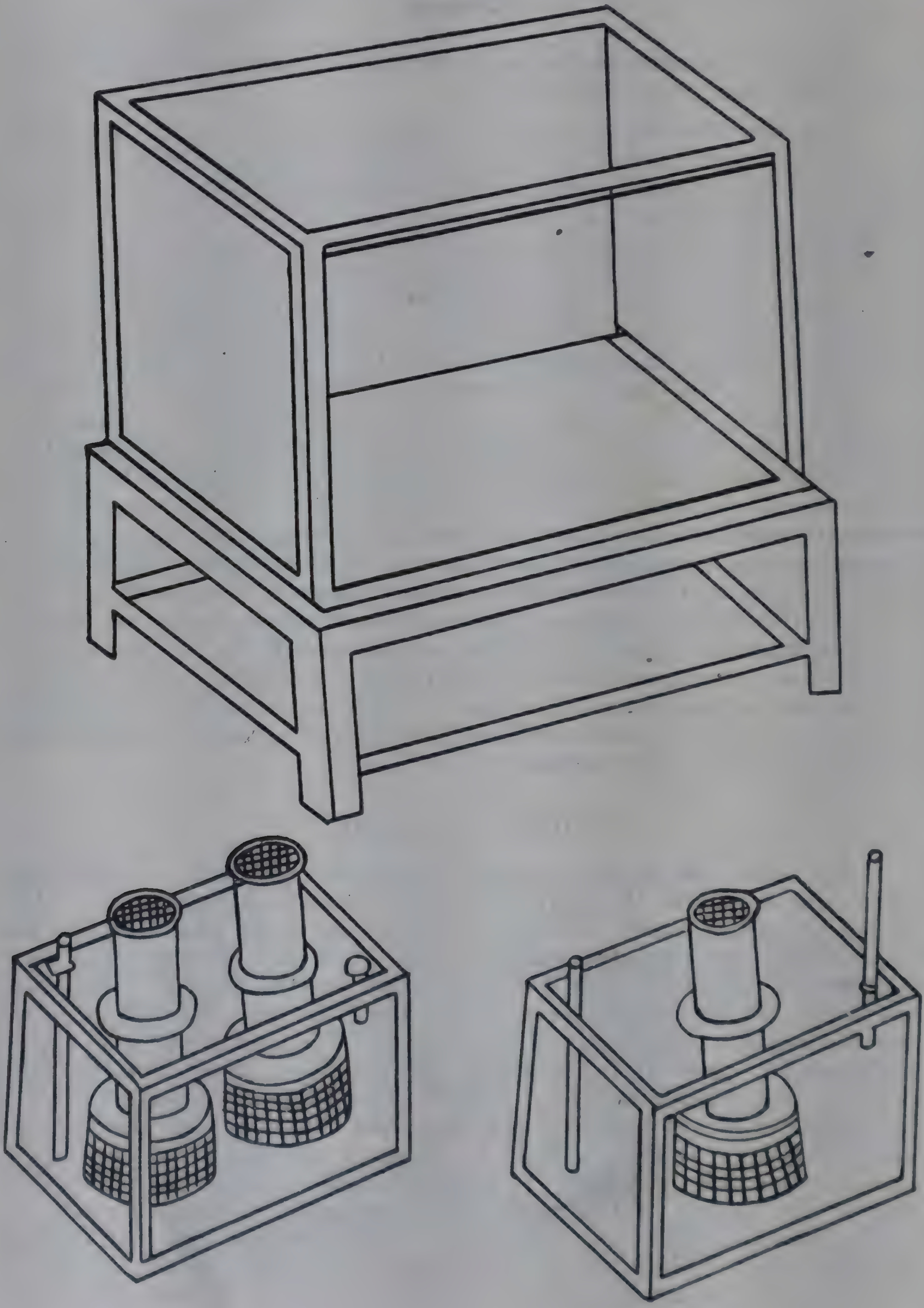
This report represents the work carried out during the period of five months beginning from May 1955.

Although attempts have been made from time to time to explain the periodic rise of plague as observed in the endemic areas in India and the carry over of infection from one plague season to another, no completely satisfactory explanation has yet been found. While there is no doubt that the periodicity of plague in a locality is governed largely by the number of susceptible rodents available, as provided by the young ones born during the interepidemic period, this hypothesis, advanced by the Russian and American workers, require rigid testing. In regard to the rôle of smoldering sylvatic plague the Indian workers, such as Sharif, Francis and Rao did not find any evidence to confirm it. In regard to the persistence of infection in the commensal rats the workers were divided into two main camps, one—believing in the existence of chronic or mitigated plague in commensal rats which could re-establish acute infection in rats or could spread the infection to others and the other not supporting this view. On account of these divergent opinions on the subject the Sub-Committee of the Plague Advisory Committee which reviewed the position in 1950 and 1954, recommended further investigation of the problem. The purpose of this present investigation was, therefore, to find out the mechanism of persistence of infection among commensal rodents and fleas which would lead to the periodic recrudescence of plague without importation. Involved in this study are the question of persistence of infection in fleas, existence of chronic plague in domestic and wild rodents, change of virulence of the plague organism, immunity in rats in different seasons, etc.

WORK SO FAR DONE.

(*a*) *Device for continuous natural transmission experiment.*—A convenient device was made for the purpose of safe transmission experiment which needed no special room. It consisted of two parts, the glass chamber and the experimental cage enclosed in a glass box (*see* sketch). The glass-chamber was made out of an *Unused aquarium* fixed on a stand. This tank was lined with glass-plates on all sides except the top. The experimental cage was made on the pattern of Gauthier and Raybaud (1902) with certain modifications. Instead of enclosing the wire-mesh cages into an open glass-box, the latter was made a closed one with top and bottom made of tin sheets. The top had four openings, two small ones on the sides for fixing ventilation tubes, about 1" in diameter and two large openings to introduce the wire-mesh cage. The use of muslin or cloth piece and of the tray at the bottom of the cages were completely avoided. Instead, the bottom was thinly layered with sand. The wire-cage had metal collars lined with felt to fit into the opening through which the cage was introduced into the box. The lid of this cage and the openings of the ventilation tubes were all covered with very fine copper wire-mesh. To prevent the rats damaging this fine wire-mesh in the lid secondary guards, made of strong wire-mesh, were fixed on its either side. The glass box with the cages were then placed inside the glass chamber. For different kinds of experiments glass boxes with single cage as well as double cages were made. In fact, if the glass chamber is big enough several experiments can be run at a time.

SKETCH.



(*b*) *Experimental observations prior to April*, 1955*:*

Experiments 1 *and* 2.—Preliminary cage experiments were carried out with both *X. astia and X. cheopis*. With the former, transmission was successful up to the second batch only when a large number of fleas as introduced into the glass chamber containing an infected mouse in one cage and a healthy mouse in the other. The interval between the death of the infected mouse and the death of the first healthy mouse was nine days and between the latter and the second healthy mouse 17 days. The third batch introduced after the death of the second, remained alive and was sacrificed at the end of 30 days period but no evidence of infection was found either in the mouse or in the fleas when examined by microscopic, cultural or animal inoculation tests. Experimentation with *X. astia* fed on infected mouse was not successful.

Experiments 3 *and* 4.—With *X. cheopis*, transmissions through fleas fed upon infected mouse and through fleas left in the glass-box with infectd and healthy mice were readily successful. It was then decided to carry out a continuous series of natural transmission with *X. cheopis* only, in the glass-cages and to watch its effect. Two series of experiments were run with the following results.—

Experiments 5 *and* 6.—In experiment 5 (duration 44 days from 12–1–54) the transmission was successful only up to the 2nd batch but the third mouse which remained alive was sacrificed and found positive for *P. pestis* both culturally and by animal inoculation, but it was of low virulence. In experiment 6, (duration 112 days from 25-2-54), therefore, organism of high virulence was chosen and interesting results were obtained. The intervals between the deaths of successive mice were at first shortened up to five days and then gradually prolonged up to 42 days in the seventh batch. After the death of the fifth batch (M6) on 6–5–54 three infected fleas were found on its body. Unlike the original organism those isolated from these fleas were serologically positive to both *P. pestis* and *P. pseudotuberculosis* antisera but giving doubtful reaction against Meyer's Plague Antigen I antiserum, indicating that the organism had already undergone considerable deterioration in virulence. This was corroborated by inoculating a pair of mice with one million organisms each on 14–5–54. One of them died on 18–6–54 (35 days after injection) and the other on 13–7–54 (60 days after). The former showed *P. pestis* in its heart's blood and spleen but no organism could be seen from the organs of the latter. There is thus an indication that the former mouse could overcome the initial onslaught of infection though given in very large dose (m.l.d. being 15 to 30 organism) but the infection persisted with development of bacteræmic condition before death. The other mouse was able to overcome the inflection completely and the death was non-specific.

The sixth batch mouse having remained without any symptoms till 21–5–54 and no flea being visible in the cage, five uninfected fleas were put in along with a normal mouse on the adjoining cage. This seventh batch mouse died on 11–6–54 and *P. pestis* was found in its heart's blood as well as in the splenic tissue. The sixth batch mouse was then still alive but became gradually sick and emaciated and died on 18–6–54, 42 days after it was placed in the cage. Postmortem: it also yielded *P. pestis* on culture. This observation also supported the findings obtained by artificial inoculation with the organism isolated from the fifth batch mouse (M_6).

The above findings led to the hypothesis that the organism got deteriorated as the epidemic proceeded and the infection was maintained for a prolonged period in the infected animal and that it was possible for the fleas to imbibe the infection from the bacteræmic condition that developed in the animal and to transmit it to another susceptible host. The fluctuation of resistance in the animal itself might also play a definite rôle in this phenomenon.

(*c*) *Experimental observations after April* 1955:

Experiments 7 *and* 8.—It was planned to carry out two series of experiments, one with the susceptible local rodents and the other with artificially immunized group of mice or rodents. Accordingly, experiments were started with two species of rats—*R. rattus* and *Bandicota bengalensis* (*G. various*), parallel with the mouse experiment, shown as under:—

EXPERIMENT 7.

Dates	*Duration of Experiment*	*Procedures and results*
16–5–55	6 days	Mouse infected with *P. pestis* placed in cage with fresh *X. cheopis*.
21–5–55	4 days	Mouse died. *P. pestis* ++ deflead Bandicota bengalensis (1) introduced with fresh fleas.
24–5–55	7 days	Tail blood of B.b.1 shows *P. pestis* + B.b(1) died.
25–5–55		*P. pestis* + B.b(2) introduced with fresh fleas.
1–6–55	6 days	B.b(2) died *P. pestis* + B.b(3) introduced with fresh fleas.
7–6–55	15 days	B.b(3) died *P. pestis* + B.b(4) introduced with fresh fleas.
22–6–55	16 weeks	B.b(4) died *P. pestis* + B.b(5) introduced with no fresh fleas.
14–10–55		B.b(5) still not dead. The mouse was killed. Postmortem splenic smear showed a few isolated group of bipolar organisms and culture positive of *P. pestis*. Experiment concluded here.

EXPERIMENT 8.

18–5–55	5 days	Mouse infected artificially placed in cage with fresh *X. cheopis*.
23–5–55		Mouse died. *P. pesits* + Splenic smear injected to deflead *R. rattus* (1).
26–5–55		*R. rattus* tail blood shows *P. pestis*.
27–5–55	5 days	Rr(1) died the same night. Rr(2) introduced with fresh fleas.
1–6–55	3 days	Rr(2) died. *P. pestis* + Rr(3) introduced with fresh fleas.
4–6–55	9 days	Rr(3) died. *P. pestis* +.
13–6–55	21 days	Rr(4) died. *P. pestis* Rr(5) introduced with fresh fleas.
4–7–55	12 days	Rr(6) died. *P. pestis* + Rr(7) introduced with fresh fleas.

16–7–55		Rr(7) died. *P. pestis*+gap of six days.
22–7–55		Rr(8) introduced with fresh fleas.
	24 days	
16–8–55	17 days	Rr(8) died. *P. pestis*+Rr(9) introduced with fresh fleas.
2–9–55	21 days	
23–9–55		Rr(9) died. *P. pestis*+Rr(10) introduced with 20 extra fleas.
30–9–55		Rr(10) died. *P. pestis* not found, fleas present. Rr(11) introduced without any extra fleas.
14–10–55		Rr(11) still alive.

The trend of results obtained in the above experiments is exactly similar to that of the experiments carried out previously with mice.

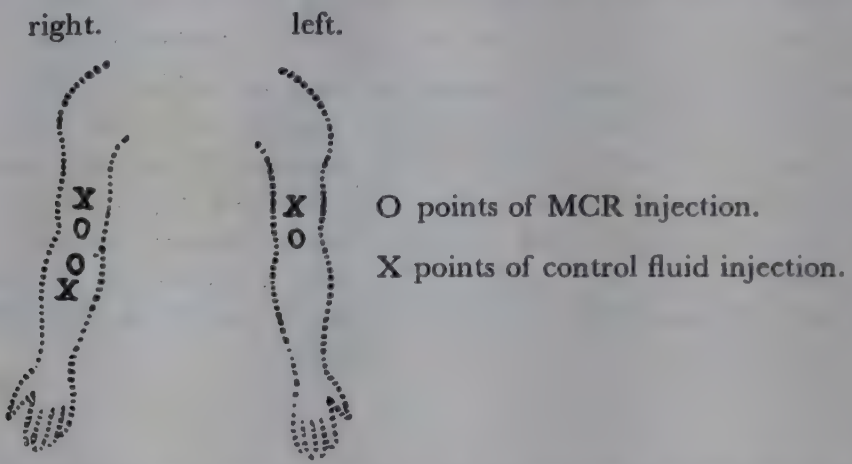
Testing of immunity in the experimental rats which will survive the infection transmitted by fleas.—For the purpose of testing the immunity level of certain experimental rats, production of several types of antiserum is in progress.

Experiments with artificially immunized mice or rodents.—This experiment will be carried out with immunized rodents first to ascertain whether chronic plague foci arise by infecting them with varying doses of virulent plague organism and later subject them to the same kind of experiment as naturally infected susceptible mice, to see whether these animals are also capable of transmitting infection at any stage. For the purpose of immunizing rats vaccine has been prepared in casein hydrolysate medium and immunization of certain commensal rats has been started.

CLINICAL RESEARCH

1. Clinical Research Unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

(1) The evaluation of MCR test was particularly of interest because of its simplicity and the reported high reliability value (95 per cent) in the early diagnosis of cancer. The test is based on the antigen-antibody reaction principle. The antigen is isolated either from gastric cancer tissue or from chorionic villi. The antigen in either case is shown by Dr. Matsubara to produce similar erythema. The two tissues are also shown to have similar glycolytic action and the same rate of respiration. A solution (0·2 c.c.) of the antigen is injected intradermally on the forearm and upper arm along the middle line of the flexor surface near the elbow joint at three points marked (0) as shown in the diagram below:


right.
left.
X
O
O
X
X
O
O points of MCR injection.
X points of control fluid injection.

Then precisely 0·2 c.c. of the control fluid is injected intradermally at each of the three points marked (x), 3 cm. away from the MCR solution injected spots. The reaction is determined by measuring the maximum diameter of the erythema at the fourth hour after injection. The limits of erythema as givn by Dr. Matsubara are as follows:—

Remarks	Control erythema, mm.	Test erythema, mm.
+ve to cancer ...	9 × 9	28 × 24
—ve to cancer ...	9 × 10	18 × 19
Indeterminate ...	14 × 13	25 × 21

The test was run on 19 healthy reaserch workers from this institute (12 males and 7 females, ranging in age between 25 and 45 years.

None of the 70 cancer patients had undergone either *x*-ray or other treatment for cancer. They had cancer at diffrent sites. Sixty-five had a positive biopsy report and the rest had clinical or radiological evidence of cancer. Twelve cases with evidence of metastasis were intentionally included in the present studies, in order to find whether these gave a negative result as claimed by Dr. Matsubara or not. The distribution of cases according to sites of cancer was, 48 (oropharyngeal region); 16 (cervix); 2 (leg); and one each of rectum, esophagus, breast and vaginal wall. Results of the MCR test are given below:—

TABLE I.

Number	Type of cases	Number of cases	Positive reaction	Negative reaction
1	Healthy adult controls	19	*Nil*	19 (100 per cent)
2	Cases with +ve biopsy and without clinical evidence of metastasis ...	53	29 (54·7 per cent)	24 (54·3 per cent)
3	Cases with +ve biopsy and with clinical evidence of metastasis ...	12	5 (41·6 per cent)	7 (58·4 per cent)
4	Cases with clinical or radiological evidence of cancer	5	3 (60 per cent)	2 (40 per cent)

Note: The area of erythema admeasuring over 300 sq. mm. was taken as positive reaction.

All the controls gave a negative reaction without a single false positive; while of the 53 cases with +ve biopsy, 29 gave a positive reaction. Of the 12 cases with metastases, 5 gave a positive reaction, the rest 7 being negative.

The test thus gave a positive result in 54·7 per cent of early cancer cases and hence cannot be said to be reliable to the extent claimed by Dr. Matsubara. However, the absence of false positives suggests that it may be useful as a diagnostic adjunct in cancer study.

(2) *Studies in phosphatase activity:*

Clinical.—The phosphatase activity has been estimated in a total number of 183 cases including 71 cases reported last year. The results obtained in 160 cases have been represented in Table II, while the results from the remaining 23 cases have been omitted because of the lack of clinical and radiological evidence in these cases.

It will be seen from Table II that the phosphatase activity was high only in three out of ten histologically-confirmed gastric cancer cases. Although this fact suggests that the increased phosphatase activity of gastric juice cannot be regarded as a reliable index of gastric malignancy, the increased phosphatase activity in all the ten cases with radiological evidence of cancer indicates a need for studying more cases of histologically proved gastric carcinoma, before arriving at any final conclusion regarding the diagnostic value of the test. From amongst the 140 cases, without any evidence of malignancy, 44 showed an increased phosphatase activity. This may be due to the absence of free HCl in the fasting samples of gastric juice in these cases.

TABLE II.

Number	Disease	Total number of cases	Number of cases with phosphatase activity		Cases with phosphatase activity above 10 & presence achlorhydria
			Below 10 K.A.U.	Above 10 K.A.U.	
1	Histologically proved cancer of stomach ...	10	7	3	3
2	Clinically and radiologically diagnosed cancer of stomach ...	10	...	10	7
3	Gastroduodenal ulcer ...	41	29	12	7
4	Gastroptosis ...	22	16	6	3
5	Pyloric obstruction ...	7	4	3	2
6	Cases with no gastric abnormalities according to G.I. series ...	70	47	23	16
	Total	160	103	57	38

Since the gastric juice is a secretion of the cells of gastric mucosa, it was considered likely that an increased enzyme activity in gastric mucosa would be reflected in the gastric juice. Kabat and Furth (*Amer. Jour. Path.*, **17,** p. 303, 1941), however, have found through histochemical studies, that the alkaline phosphatase is totally absent in normal and cancerous gastric mucosa. In view of these facts, a study of the enzyme activity in normal and cancerous gastric tissues was considered worth investigating.

So far five normal and six pathological tissues have been studied for their enzyme activity. Normal tissue was obtained along with the excised pathological tissue and served as control for the same. Histological confirmation was obtained for both normal and diseased part of tissue.

TABLE III.

Showing acid and alkaline phosphatase values in human normal and pathological gastric tissue.

Number	NORMAL TISSUE: Phosphatase activity/100 g.		Diagnosis	PATHOLOGICAL TISSUE: Phosphatase activity/100 g.	
	Alkaline	Acid		Alkaline	Acid
1	...	...	Adenocarcinoma Gr. II	208·5	612·8
2	72·0	562·0	,, ,, ,,	139·0	587·0
3	72·4	274·0	Adenocarcinoma colloid type	505·3	418·4
4	80·5	396·6	Adenocarcinoma ...	72·36	307·5
5	87·3	718·8	Chronic gastric ulcer	172·2	441·6
6	67·7	576·3	,, ,, ,,	682·6	709·7

It can be seen from Table III that the alkaline phosphatase is present in the gastric tissue and that its activity is increased in pathological tissue. The average values for alkaline and acid-phosphatase activity are 76·0 and 505·5 K.A. units per 100 g. of tissue, respectively. The number of tissues so far studied being very small, no final conclusions regarding the phosphatase activity can be drawn at this stage.

(3) In this laboratory, the surgical technique of Toovey *et al.* (*Canc. Res.*, **11,** p. 912, 1951) is used and the stomach flaps are being painted with 0·6 per cent solution in benzene of 20-methyl-cholanthrene, in view of the observation of Stewart *et al.* (*Jour. Nat. Cancer Inst.*, **10,** pp. 359-60. 1949) that gastric adenocarcinoma could be produced by intramural injection of 20-methyl-cholanthrene. For establishing the normal levels of enzyme activity in gastric mucosal flaps, the animals were divided into three batches and were sacrificed at the end of 10, 30 and 52 days after operation. So far 13 normal flaps have been studied and the results are given in Table IV.

TABLE IV.

Showing the enzyme content of gastric flaps.

Batch number	Animals sacrificed on:	Number of animals	PHOSPHATASE ACTIVITY K.A. UNITS/G.	
			Alkaline	Acid
1	10th day after operation ...	6	12·85 ± 0·97	6·86 ± 0·7
2	30th day after operation ...	6	13·00 ± 1·51	8·38 ± 0·81
3	52nd day after operation ...	1	10·3	21·50

The experimental rats for the induction of gastric carcinoma have also been divided into three groups, viz., (*a*) normal control, (*b*) benzene control, and (*c*) methyl-cholanthrene painted ones. The animals are painted with the solvent and the carcinogenic solution on alternate days.

2. Neuropathological Unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

I. EXPERIMENTAL STUDIES IN LATHYRISM.

(*a*) Experiments involving feeding of *Lathyrus sativus* in proportions of 70, 90 and 100 per cent, respectively, to Wistar rats referred to in our last report were brought to completion. As was indicated in the last report the only clinical difference between these animals and their respective controls, was a slightly greater gain in weight in the controls. Complete histological examination of viscera, muscle, nerve, and nervous system of a representative proportion of animals from all the groups on lathyrus diet has been performed. In most of the animals maintained on lathyrus scattered degenerative changes with glial reaction were seen in the cerebral cortex, basal ganglia, brain-stem tegmentum, and anterior horns of the spinal cord. Control animals maintained on stock diet for the same period revealed similar changes but to a lesser degree. In both the groups there were no significant changes in the fibre columns of the spinal cord.

(*b*) Two *rhesus* monkeys maintained on a 70 per cent lathyrus diet for about 21 months were sacrificed for histological examination. These were the animals that remained out of the four started on this diet in 1953. Both these animals revealed a moderate to marked hyperplasia of the R.E. cells in the liver, and deposition of an amorphous pink staining material in the malpighian follicles of the spleen. The brains and spinal cords were studied by a method of incomplete serial sectioning. Focal outfall of neurons, degenerative changes in nerve cells in the cerebral cortex, basal ganglia (thalamus and globus pallidus), brainstem tegmentum, and anterior horns of the spinal cord accompanied by appropriate glial reaction was encountered in both these animals. Comparison was made with identically stained sections of a normal *rhesus* monkey. As in the rats no noteworthy changes were encountered in the white fibre columns of the spinal cord and peripheral nerves.

(*c*) The repeated failure in the attempted (experimental) induction of a disease comparable to human lathyrism in laboratory animals, by feeding of lathyrus alone necessitates a reconsideration of the possible pathogenesis of this disorder. One of the possibilities thought of is that while lathyrus alone may be inadequate to induce lathyrism, the feeding of lathyrus for a sufficient length of time, may so condition the nervous system that it reacts with facility to subminimal doses of some other neurotoxic agent. An experiment has been planned to test out this hypothesis using rats as the experimental animals and sodium azide as the test toxic agent. Sodium azide was chosen because of the similarity of its action on the nervous system to cyanide. Both these agents are metabolic inhibitors and with repeated administration damage specifacally the white matter of the central nervous system (Hicks, *Arch. Path.*, **50**, p. 545, 1950).

The general mode of procedure was to feed *Lathyrus sativus* in high proportions (90 per cent and 100 per cent) to animals along with suitable controls on stock diet. Sodium azide would then be administered to a proportion of the lathyrus-fed and stock-diet animals, in repeated daily doses (preliminary trials indicate that the majority of animals, tolerate 10 mg./kg. body weight daily for at least seven days without any demonstrable clinical effects). After a specified number of injections all the animals would be sacrificed and comparison made of the lesions in the nervous system of the four groups. The experiments along these lines possible so far appear to suggest that lathyrus with azide produces comparatively more of neuronal and glial changes than lathyrus alone, or azide alone. As this study had to be restricted it is, however, considered desirable to use a larger number of animals as also other species particularly monkeys, and other known neurotoxic agents, before any definite statements can be made.

II. Nerve and muscle in leprous polyneuritis.

This clinico-pathologic study was completed this year. A total of 75 muscle biopsies were collected, but 11 of these obtained from the tibialis anterior muscle are excluded from consideration for the present. The following is a summary of the sensory and motor features and the histological findings in 64 of the cases, who provided a muscle biopsy from the superior extremity—usually from the flexor carpi ulnaris muscle.

(*a*) *Clinical features.*—A variable degree of thickening of both the ulnar and lateral popliteal nerves was noted in the majority of cases. On examination of motor power and muscle function, these cases could be classified into three groups.

(*i*) *The unaffected group:* (17 cases).—In these, one of the upper limbs from where the biopsy was obtained revealed normal motor power and muscle function. In six of the 17 the nerve trunks were normal to palpation. The cutaneous sensations were unaffected in nine cases, and lost or impaired in the area of distribution of the ulnar nerve and/or the medial cutaneous nerve of the fore-arm in the others.

(*ii*) *The moderately affected group:* (17 cases).—Here the muscle disability was usually restricted to a weakness of abduction—adduction of the little or little and ring fingers. Good contraction of the flexor carpi ulnaris muscle was elicited in all. Some degree of fixed flexion of the distal phalanx of the fourth and/or fifth digits was noted in a few instances. Cutaneous sensibility was lost in eight cases and impaired in eight cases, usually over the zones of innervation of the ulnar and the medial cutaneous nerve of the fore-arm, but occasionally of the medial nerve also.

(*iii*) *The severely affected group:* (30 cases).—All these were characterized by extreme weakness or paralysis and wasting of most of the intrinsic muscles of the hand. Abduction and adduction of the two medial fingers was hardly possible in any case, and was restricted even for the lateral fingers and the thumb in 16 cases. Limitation of flexion and extension at the meta-carpo phalangeal and interphalangeal joints was observed in 12 cases. Weakness of the flexor carpi ulnaris muscle was present in all the 30 cases and almost total loss of contraction in 13 of these. In four, ulnar deviation or extension of the wrist was markedly affected.

Cutaneous insensibility in the area of distribution of the ulnar and median nerves and the medial cutaneous nerve of the fore-arm was noted in all 30 cases, and of the radial nerve also in about half of these (for the actual areas refer to 'Aids to the Investigation of Peripheral Nerve Injuries'. *M.R.C. War Memorandum No.* 7, London, 1943).

Contractures of the fingers were observed in all members of this group, 24 of the 30 presenting a claw-hand. Absorption of one or more of the terminal phalanges was sometimes encountered, but less frequently than that of the toes. The presence of contractures and mutilations appeared to bear a direct relationship to injuries suffered by the hand, and many of these subjects revealed healed and unhealed scars—especially on the fingers.

(*b*) *Morbid anatomy and histopathology.*—The three components of the lower motor neuron known to be affected in leprosy are, proceeding centrifugally, the nerve trunk, the intramuscular nerve terminals and the striated muscle.

(*i*) *The nerve trunk.*—Portions of one thickened ulnar nerve and flexer carpi ulnaris muscle obtained at autopsy (from a case with leprous neuritis of all four limbs) were examined after staining with Masson's trichrome, Mallory's aniline blue, and Fite-Faraco's carbol-fuchsin techniques.

There was general preservation of the normal funicular pattern of the whole nerve. In places a disruption of its finer architecture by scattered lepromatous exudates was seen along with the usual reactive and degenerative changes evoked by the acid-fast bacilli which were found in singles among the nerve filaments and in clusters in lepra cells. The muscle showed distinct atrophic changes.

(*ii*) *The intramuscular neural elements.*—These were clearly visualized in whole mounts of muscle tissue stained vitally with methylene blue, if the biopsies were performed at or near the ' motor point ' of the muscles concerned. This was fairly constantly achieved in the case of the flexor carpi ulnaris muscle which provided the biopsy in almost all of the 64 ' cases ' under analysis.

The general pattern of intramuscular nerve fibres and endings clearly resembled that described by Feindel, Hinshaw and Weddell (*Jour. Anat.*, **86,** p. 35, 1952) in the rabbit, and by Coers (*Jour. Neurol. Neurosurg. Psychiat.*, **15,** p. 311, 1952) in man. Interfascicular nerves, branches, pre-terminal fibres, motor end-plates, spindles and sensory nerve endings could all be observed.

The pathological changes in the general pattern of motor innervation were reflected in all its components. The interfascicular nerve or its branches ran a devious course and their constituent fibres tended to be irregular in size and arrangement, especialy in the presence of perineural infiltrates. Beading and fragmentation of the axons and proliferation of schwann nuclei and blood capillaries indicated degenerative changes. These were accompanied by regenerative phenomena, such as the sprouting of delicate new nerve filaments collaterally from the remaining intact fibres, and the formation of terminal ' growth cones ', as in the cutaneous lesions (Dastur, 1955, *Brain*, in press).

The normal ' spray' of pre-terminal fibres was often disrupted and reduced and the motor endings either disappeared or assumed bizarre, usually shrunken forms or, rarely, remained normal. Attempts at regeneration of the end-plates were infrequent and resulted in the formation of fragile, rudimentary structures with just two to three ' nuclei '. The nerve fibres of the muscle spindles also exhibited degenerative changes such as those indicated above, but the intra-fusal muscle fibres invariably appeared unaffected (Batten, *Brain*, **20,** p. 138, 1897). The majority of specimens of the severely affected group of cases revealed no nerve fibres at all. On the whole, the quantitative alterations in the intramuscular neural organization were more significant (Table I) than the qualitative changes.

It is apparent from Table I that any neurohistological difference between the unaffected and the moderately affected groups of cases is insignificant, while between either of these and the severely affected group, there is a striking difference. This is most pronounced when end-formations (end-plates and spindles) are considered. This would indicate that in leprous neuritis increasing grades of muscular disability are concurrent with increasing degrees of intramuscular neural degeneration; and that the nerve terminals are affected earlier and more severely than the nerve bundles. It would also appear that morphological damage assumes, in this type of leprosy, significant proportions only in the very late stages. The presence of fairly long periods during which slowly progressive and probably reversible changes are alone likely to be manifest, is obviously of practical importance from the point of view of treatment and prognosis.

TABLE I.

Quantitative analysis of neural histology and its correlation with clinical findings.

Motor status, clinically	Un-affected	Moderately affected	Severely affected	Total
Number of muscle biopsies ...	17	17	30	64
Number showing nerves only	16	13	12	41
Number showing end-formation also	12	10	6	28
Number showing qualitative changes	4	8	4	16

It is not surprising that half the number of cases revealing degenerative and/or regenerative changes (8 of the 16—Table I), belonged to the moderately affected group.

(*iii*) *The striated muscle.*—Valuable information was gained from simple H.E. stained sections of small bits of excised muscle that could be spared for this purpose.

The microscopic examination of skeletal muscles from most of the unaffected group of cases revealed normal structure. In the other two groups all grades of neural muscular atrophy were observed (‘*Diseases of Muscle*’, Adams *et al.*, Paul B. Hoeber Inc. 1953). Fatty or fibrous replacement of muscle was not observed.

TABLE II.

Quantitative analysis of muscle histology and its correlation with the clinical findings.

Motor status, clinically	Un-affected	Moderately affected	Severely affected	Total
Number of muscle biopsies ...	15	17	28	60
Normal or very slightly affected muscle	12	8	8	28
Moderately and severely affected muscle	3	9	20	32
Regenerating muscle ...	...	...	6	6

Table II indicates a clear gradation of pathological changes from the clinically unaffected, through the moderately affected to the severely affected group of cases. Secondly, it is seen that over half the muscle specimens of the moderately affected group of cases (9 of the 17) manifested definite degenerative changes, even though their overall content of nerve fibres and endings matched that of the unaffected group (of Table I). This would imply that the atrophic change is revealed first by the most distal component of the motor unit—the muscle fibre, degeneration of the terminal motor nerve and end-plate following in its wake.

III. THE EFFECT OF REPEATED EPISODES OF INDUCED HYPOGLYCEMIA ON THE CENTRAL NERVOUS SYSTEM OF THE MONKEY.

It is well known that for its energy requirements, the brain *in vivo* is almost entirely dependent on oxidation of glucose. Withdrawal of this substrate or lowering the rate at which it is delivered to the brain gives rise to a series of phenomena, the complexity of which appears to be related to the functional organization of the nervous system. As a result of the introduction of the well-known insulin treatment in schizophrenia it has become possible to study the pattern of the neurological reaction during hypoglycemia in man. For obvious reasons any planned study in such subjects can never be complete. While the general pattern of the neuropathological changes have been briefly described in human beings and a few other animal species adequate information is not available regarding the level and duration of hypoglycemia in relation to the production of irreversible damage to various regions of the brain. The following study in monkeys was, therefore, undertaken in an atempt to (*a*) Study the entire pattern of the neurological response to hypoglycemia and to correlate the various phases of this response to the levels of the circulating blood sugar, (*b*) follow up with a complete histological and, if possible, histochemical and biochemical study of the brian, and (*c*) study the late effects of severe hypoglycemic episodes.

Adult *rhesus* monkeys weighing between 10 and 12 lb., starved for the previous 24 hours, were rendered hypoglycemic by the subcutaneous injection of soluble insulin. A pre-injection blood sample, and samples thereafter at intervals of 1 to 1½ hours and/or during the various phases of the episode were obtained for glucose estimation and leucocyte counts. A typical episode is summarized in Table III:—

TABLE III.

Monkey starved for 24 *hours. Fasting blood sugar* 80 *to* 120 *mg.*/100 *c.c.*

Time intervals:		Clinical features	Range of blood sugar mg./100 c.c.	Leucocytes count/ cu. mm.
Hours	Minutes			
00	00	Animal quite active and alert. Insulin administered subcutaneously ...	80–120	12,000
01	30	Spontaneous activity less. Animal alert. Head droops...	About 40	25,000
02	30	General muscle weakness. Animal supports itself. Transient grasp ...	About 30	30,000
03	30	Muscle weakness++Animal lies down. Pupils dilate. Eye-brows twitch. Occasional extensor plantar ...	About 25	35,000
04	00	Convulsions supervene, first tonic, followed by conic with centripetal progression	About 15	35-40,000
04	05	Momentary recovery; animal alert. Grasp and extensor plantar persist ...	About 40	...
04	15	A second convulson and torsion spasm or mere prostration	About 15	...
04	30	Animal totally unresponsive. Commencing decerebration; tonic neck reflexes	About 15 or less	...
Later		Phasic or shallow breathing; total unresponsiveness persists. Revived with I.V. glucose: unsteadiness for variable periods, transient amaurosis ...	About 10	35-40,000

With such episodes repeated within a short time of one another, the following features have been observed:—

(*a*) It is necessary to increase the amount of insulin administered each time in spite of varying the site of injection.

(*b*) In the later episodes the entire picture may not be presented and some of the earlier phases may be entirely misssed.

So far about 20 full episodes in three monkeys have been correlated with blood sugar findings. All the animals have been autopsied and await histological examination.

IV. STUDIES IN PYRIDOXINE AND PANTOTHENIC-ACID DEFICIENCIES.

The comparative effects of pyridoxine and pantothenic-acid deficiencies on the nervous system of swine were studied by Follis and Wintrobe (*Jour. Exper. Med.*, **81,** p. 539, 1945). These studies appear to indicate that the primary injury to the neuron in pyridoxine deficiency is in its peripheral process, while in pantothenic-acid deficiency the initial injury appears to be in the cell body. Swank and Adams (*Jour. Neuropath. Exper. Neurol.*, **7,** p. 274, 1948) do not agree to this specificity of site.

The pattern of changes in neurons in these deficiency states would be of special interest in view of the two main types of lesions encountered in the nervous system, viz., myelinoclastic and polioclastic. It was, therefore, considered worthwhile to study the effects of induced deficiencies of these vitamins in monkeys.

Two monkeys were fed synthetic diet (Waisman and McCall, *Arch. Bioch.*, **4,** p. 265, 1944) incorporating all the known vitamins including pyridoxine for 2½ months. These animals served as their own controls before they were transferred to the experimental diet in which only the pyridoxine was withdrawn from the synthetic stock diet. The progress of the deficiency is being followed by examining the blood level of pyridoxine (by microbiological assay using saccharomyces carlbergensis as the test organism) at intervals of two to three weeks. Following three months of feeding on such a diet the level of pyridoxine in two monkeys has stepped down from the basic values of 28·0 μg. and 29·6 μg. to 7·5 μg. and 5·0 μg. per 100 c.c., respectively. These levels have been correlated certain relevant data, such as hæmoglobin, urea and plasma total lipids, cholesterol and iodine number of fatty acids. It is proposed to continue the experiment until clinical symptoms of deficiency are precipitated when the animals will be sacrificed for histology. A detailed account of the histological and biochemical picture will be given at a later date.

V. REFERENCE WORK.

As in previous years a number of brain and muscle biopsies and material from human and animal neuropathological autopsies are referred to the Unit for opinion. The following material was received for study during the period under report:—

(*a*) Sixty-two space-occupying lesions of the C.N.S. The majority of these were neoplasms (85 per cent) the rest were constituted of productive granulomata (15 per cent).

(*b*) Twenty-five muscle biopsies were received; 17 of these were neural atrophies and 8 muscle dystrophies.

(*c*) Complete brains from two rare neurological disorders were obtained from the Department of Neurology, K.E.M. Hospital. The first was a case of Jakob-Creutzfeld disease and the second was one of febrile myoclonus with E.E.G. changes of subacute progressive encephalitis. Both these brains are in the process of being studied by a method of incomplete serial sectioning.

(*d*) Neuropathological material was received from two cases of caprine paralysis form an epidemic in Cuttack, Orissa. One of these revealed lesions consistent with helminthic encephalitis.

(*e*) *Virus encephalitis.*—A complete half brain and portions of viscera from a typical fatal case of 'virus encephalitis' was received from the Irwin Hospital, New Delhi. The striking feature of this case was a severe degree of fatty metamorphosis of the liver and kidney. Neurons of all levels in the brain revealed alterations characteristic of acute cell damage without any corresponding glial or vascular reactions. The findings were similar to those encountered in the previous autopsies of 'encephalitis' studied by this Unit.

3. Inquiry on the rôle of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

1. AMINO-ACID COMPOSITION OF HUMAN MILK.

Essential amino-acids content of 17 samples of milk of mothers belonging to low economic groups, determined by the microbiological-assay technique, was reported last year. This work was further continued. In addition, preliminary data regarding the quantitative yield of milk were also collected from mothers in three local institutions.

During the period under report, 44 more samples of milk were analysed. In order to study the essential amino-acid pattern at different stages of lactation, these samples are classified arbitrarily into the following five groups, depending upon the period of lactation: one to five days, six days to three months, four to six months, seven to twelve months and over twelve months. The number of samples in each group are 5, 6, 14, 17 and 2, respectively.

The values for most of the amino acids in the samples of milk analysed are in agreement with those reported before. When these values are expressed on the basis of 100 c.c. of milk, there is a considerable drop in the values immediately after the cessation of colostrum secretion. Following this period, the levels of the amino acids remain practically constant. On the other hand, when the amino-acid values are expressed on the basis of 100 g. protein, there is a marked variation at different periods of lactation, though the total nitrogen is practically constant after the initial period of five days.

In order to study the quantitative yield of milk in mothers belonging to low economic groups, test feeds were carried out in 45 cases. The child was weighed before and after each feed during 24 hours. The tests were carried out in three centres and the results are given in Table I:—

TABLE I.

Number	Name of the centre	Number of cases	AGE OF CHILD:	TOTAL YIELD OF MILK PER DAY IN OZ.	
			Range	Range	Mean
1	Nowrosjee Wadia Maternity Hospital, Parel,-Bombay-12	25	1—17 days	7 to 25	13·86
2	The Bombay Mothers & Children Welfare Society, Delisle Road Maternity Home, Bombay-11 ...	15	2—6 days	6 to 20	12·77
3	The Tata Mills, Parel, Bombay-12 ...	5	2—10½ months	12 to 22	17·4

Samples of milk for amino-acids analyses were received from the B. J. Hospital for Children (J. J. Group of Hospitals) Bombay-8, and the B. J. Wadia Hospital for Children, Parel, Bombay-12. Test feeds were carried out in the three institutions mentioned above. Grateful acknowledgement is made to the medical officers and the staff of these institutions for their kind co-operation.

2. INFLUENCE OF FERMENTED FOODSTUFFS ON FATTY INFILTRATION OF THE LIVER IN RATS.

It has been observed last year (1953-54) that in rats fed high-fat low-protein diet (30 per cent fat, 10 per cent casein), 'idlee' (fermented preparation) feeding at 50 per cent and 100 per cent replacement of dietary protein (casein) had beneficial effect on fatty infiltration in the liver. This was indicated both by biochemical and histological findings. The histological examination of the liver sections of the animals in the second experiment was then in progress. The observations on this material are given below:—

Liver sections of animals fed on the unfermented preparation showed fatty infiltration but no fibrosis of the liver. In case of the 'idlee' group, the liver sections showed normal lobular pattern with occasional fatty infiltration of the parenchymal cells here and there. Although the general histological picture was similar to that reported last year, where 50 per cent of the dietary protein was replaced by 'idlee' the preventive effect was not so pronounced. This is in conformity with the biochemical findings.

During the year, it was intended to confirm the above results. For this purpose, another experiment was carried out to study the preventive effect of 'idlee' on fatty infiltration. In these tests larger number of animals were used and 50 per cent of the casein was replaced by 'idlee' protein. The experiment was continued for 60 days. The other experimental details were as reported before. The results are summarized in Table II.

TABLE II.

Group	Number of rats	Number of days	Gain in weight, g.	G. PER 100 G. OF THE BODY-WEIGHT:		
				Liver weight	Liver fat	Liver protein
Control	6	60	32·9	11·06	3·3	1·14
'Unfermented preparation'	12	60	43·0	8·57	1·83	1·01
'Idlee' (fermented preparation)	12	60	58·0	6·55	1·02	0·84

This experiment confirms our previous findings—both biochemical and histological—that ' idlee ' affords greater protection to the liver than the ' unfermented preparation '. This finding is statistically significant. It is also evident that even in ' idlee ' group, a combination of protein (50 per cent casein and 50 per cent ' idlee ' protein) is superior to either of the two alone.

3. EXPERIMENTAL HEPATIC NECROSIS.

Acute massive necrosis of liver has now been produced regularly in rats fed yeast at 20 and 15 per cent levels. In order to investigate the possible metabolic changes taking place in such livers at the onset of the disease, some biochemical estimations were undertaken. To begin with water, nitrogen and alkaline phosphatase levels of livers—necrotic and normal—were determined.

One group of twelve rats was fed ' yeast necrogenic diet ' at 20 per cent yeast level, while the control group of ten rats received diet containing casein at ten per cent level in place of yeast. The level of protein in both the diets was about equal. The rest of the supplements were similar to those used in our previous experiments. The initial average weight of the animals was about 114 g.

The estimations of moisture and nitrogen in samples of liver were carried out by the usual methods. Alkaline phosphatase was extracted from fresh liver samples by the method of Davis (1934) and the rest of the procedure was similar to that outlined in Hawk *et al.* (1953) for serum phosphatase.

The rats maintained on both the ' yeast necrogenic diet ' as well as the control diet grew at sub-optimal rate, the average increase in weight at the end of two months being 34 g. and 53 g., respectively. The rats on the test diet developed hepatic necrosis between the period 60 and 165 days. The time required for the production of liver necrosis in this group was longer than before as older animals were used. However, liver necrosis was uniformly found in all the animals fed the test diet. It has been observed that the necrotic livers were heavier than the control, the average liver weight per 100 g. body-weight being 5 g. in the former, while it was 3 g. in the latter. This is evident from the water content values which are

78·4 per cent and 69·5 per cent in the necrotic and the non-necrotic livers, respectively. The estimations of nitrogen showed about 19 per cent decrease in the necrotic livers as compared to the control. The alkaline phosphatase activity of the necrotic livers showed considerable increase in comparison to that of the livers from the control animals. The average phosphorus liberated in two hours by one g. necrotic liver was 5·5 mg., while it was 1·1 mg. in case of the non-necrotic liver.

Experiments to produce massive hepatic necrosis in mice have been planned and are in progress.

4. Hæmatological Unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

I. Nutritional macrocytic anæmia.

(A) *Experimental.*

Six healthy monkeys (*M. rhesus*) with normal hæmatological values were selected for study. Their body weights varied from 9 lb. to 15 lb. Baseline studies were carried out while the animals were subsisting on the usual animal room diet.

(*a*) *Serum vitamin* B_{12}.—Vitamin B_{12} concentration of serum was determined microbiologically by the method of Ross (*Jour. Clin. Path.*, **5**, p. 250, 1952) using *Euglena gracilis* var. *bacillaris* as the test organism. The results of the assay are shown in Table I:—

TABLE I.

Vitamin B_{12} *concentration of serum in normal monkeys.*

Monkey number	Serum vitamin B_{12} concentration in micromicrogram/c.c.		Ratio
	Free	*Total*	*Free/Total*
1	28·0	42·0	0·66
2	34·0	56·0	0·60
3	40·0	50·5	0·79
4	10·0	40·0	0·25
5	41·0	94·5	0·43
6	20·0	52·0	0·38
Mean value	28·9	56·8	0·50

Mean values of free and total vitamin B_{12} were 28·9 and 56·8 u μg. per c.c., respectively. Free vitamin B_{12} on an average constituted 50 per cent total vitamin B_{12}.

Wilson and Pitney (*Jour. Lab. Clin. Med.*, **45,** p. 590, 1955) using the same assay technique, obtained mean values of 69 u μg. (range 33 to 132) and 120 u μg. (range 50 to 234) respectively, for free and total vitamin B_{12}

in 17 'apparently normal' monkeys (*M. mullata*); free vitamin B_{12} in their series constituted 58·3 per cent of the total vitamin B_{12}. Both the free and toal vitamin B_{12} levels in the present series were thus lower than those obtained by Wilson and Pitney, though the ratio (50 per cent) of free to total vitamin B_{12} in the present series conformed to that (58·3 per cent) of Wilson and Pitney.

Both the free and total vitamin B_{12} concentrations of monkey serum were definitely lower than the corresponding human values, while the ratio of the free to total vitamin B_{12} in monkey serum was considerably higher (Tables I and III).

Comment.—It is probable that the higher values of both the free and total serum vitamin B_{12} level in the series of Wilson and Pitney was due to their monkeys' 'natural' laboratory diet being higher in vitamin B_{12} content. The significance of monkey serum containing relatively greater amount of free vitamin B_{12} is not clear.

In the earlier part of the experiment the concentrations of vitamin B_{12} and folic acid in the urine were also estimated. In an endeavour to collect 24-hour samples of urine without fæcal contamination, the monkeys were placed in specially prepared metabolic cage. But in spite of all the precautions fæcal contamination of urine could not be prevented and the results of urinary examination gave widely divergent values. In view of this obvious difficulty urinary estimations were subsequently discontinued.

(*b*) *Serum folic acid.*—Serum folic acid concentration was estimated microbiologically by the method of Tepley and Elvehjem (*Jour. Biol. Chem.*, **157,** p. 303, 1945), using *Streptococcus fæcalis* R as test organism. Mean value was 0·61 millimicrogram per c.c.

(*c*) *Serum iron and iron-binding capacity of serum.*—Mean serum iron level was 87·0 microgram per 100 c.c. (range 45·0 to 135·0). Mean unsaturated iron-binding capacity of serum (U.I.B.C.) was 282·0 μg. (range 200·0 to 300·0). These values were similar to the corresponding values in man.

After completion of baseline studies, four monkeys were put on exclusive milk diet with a view to induce macrocytic anæmia as produced by May *et al.* (*Amer. Jour. Dis. Child.*, **80,** p. 191, 1950). The concentration of vitamin B_{12}, folic acid and vitamin C in the sample of boiled milk as prepared from milk powder and supplied to the animals were, respectively, 3·01 μg., 2·48 μg., 3·2 mg. per litre. The remaining two monkeys were allowed to continue the usual animal room diet and served as controls.

Effect of milk diet.—With five months of continued milk diet there was no significant change of blood picture, serum iron or iron-binding capacity of serum. Contrary to expectation, serum vitamin B_{12} level showed distinct rise, mean values for free and total vitamin B_{12} being 116·5 and 189·5 per c.c., respectively (Table II). Serum folic-acid level, however, showed no significant change.

TABLE II.

Vitamin B_{12} concentration in serum before and after milk diet.

Monkey number	Free vitamin B_{12} u/μg./c.c.		Total vitamin B_{12} u/μg./c.c.	
	Before	After	Before	After
1	28·0	...	42·0	...
2	34·0	...	56·0	...
3	40·0	160·0	50·5	225·0
4	10·0	165·0	40·0	300·0
5	41·0	68·0	94·5	120·0
6	20·0	73·0	52·0	113·0
Mean	28·9	116·5	56·8	189·5

In view of the failure of the above regime to produce any anæmia and in view of increased vitamin B_{12} levels in the experimental monkeys, the milk was further treated with copper, sulphate (20 μg. per litre) and hydrogen peroxide (0·4 c.c. 'perhydrol' per litre) to lower the concentration of vitamins in the milk. The concentration of vitamins in the milk so treated was vitamin B_{12} 0·05 μg., folic acid 0·8 μg. and vitamin C 1·5 mg. per litre. The effect of this diet which is distinctly low in vitamins is being awaited.

(B) *Serum vitamin B_{12} level in N.M.A.*

Mean values of free and total vitamin B_{12} were 15·0 (range 10 to 20) and 75·0 (range 20 to 170) micromicrogram per c.c., respectively. These values were significantly lower than the corresponding values of normal Indians. After treatment with specific anti-megaloblastic drugs the level of total vitamin B_{12} increased considerably but free vitamin B_{12} level continued to be low (Table III).

TABLE III.

Vitamin B_{12} concentration in serum.

Subjects	Number	VITAMIN B CONCENTRATION IN MICROMICROGRAM/C.C.				RATIO	
		Free		Total		Free/Total	
		Mean	Range	Mean	Range	Mean	Range
Normal Indian	14	53·3	20–164	295·5	98–600	0·18	0·083 ·276
N.M.A. (before treatment)	6	15·0	10–20	75·0	20–170	...	...
N.M.A. (after treatment)	6	16·3	10–28	163·0	100–230	...	...

The findings suggest that in an average case of N.M.A. as seen in India there is deficiency of vitamin B_{12} (Das Gupta, Chatterjea, Ghosh and Banerjee, *Bull. Cal. School Trop. Med.*, **3,** p. 101, 1955).

II. COOLEY'S ANÆMIA.

Salient features of the disease as studied in 44 patients are given below:—

Clinical.

Age.—Below five years, 24 cases, age of the youngest patient was only three months. Between five and ten years, ten cases. Above ten years, ten cases, the age of the oldest patient was 49 years.

Sex.—Males 25, females 19.

Facies.—Classical mongoloid facies was seen in 14 cases and depressed bridge of the nose in 22, while normal facies was seen in 8 cases. Normal facies *per se* did not preclude the diagnosis of Cooley's anæmia.

Liver.—Enlarged in all the cases. Just palpable to 1″ in 20 cases; 1″ to 2″ in 21 cases; and 2″ to 3″ in three cases.

Spleen.—Enlarged in all the cases. Just palpable to less than 2″ in 17 cases; 2″ to less than 4″ in 14 cases and 4″ to 6″ in 13 cases.

Other features.—Poor somatic development was present in 40 cases, but there was no impairment of intelligence and psychical faculties in any of the cases. One patient aged 13 showed evidence of sexual infantilism. The oldest patient aged 49 had a daughter aged 15 who had no anæmia but unmistakable evidences of Cooley's trait. One young female patient aged 24 had 2 pregnancies but both ended in miscarriages. Hypersplenism with pancytopenia was noted in three cases, leg ulcer in one case and cholelithiasis in one case.

Hæmatological.

Blood picture.—The blood picture was hypochromic in all cases. Hypochromicity was associated with microcytosis in five, with normocytosis in 29, and macrocytosis in ten cases. Anæmia was severe (r.b.c. below 1·5 millions) in nine, moderate (r.b.c. between 1·5 and 3·0 millions) in 26 and mild (r.b.c. above 3·0 millions) in nine cases. Reticulocyte count varied from 2·8 per cent to 37 per cent and the mean reticulocyte count was 12·3 per cent. Total w.b.c. count (corrected) was less than 5,000 in four; between 5,000 and less than 10,000 in fourteen; between 10,000 and less than 20,000 in 21; between 20,000 and 30,000 in five.

Varying degrees of erythrocytic abnormality characterized by polychromasia, Cabot's ring, Howell-Jolly bodies, basophilic stippling and the presence of varying number of normoblasts (153 to 7,715 per cm.) and target cells, were pesent in all the cases. Target cells were usually seen in greater numbers in the milder forms of the disease where normoblastosis was slight; target cells were relatively scanty in the severe forms of the disease where normoblastosis was high.

van den Bergh test.—Mean bilirubin content was 1·25 mg. per cent and the range varied from 0·2 to 2·0 mg. per cent. In infants bilirubinæmia was minimum.

Fragility of red cells: (a) Osmotic fragility.—When tested in graded hypotonic saline solution the fragility of red cells was found decreased. In an average case hæmolysis started at a concentration of 0·4 per cent solution of sodium chloride and was not complete until a concentration as low as 0·14 per cent was reached. In extreme cases complete hæmolysis occurred only at a concentration of 0·08 per cent solution. This osmotic resistance provided a diagnostic test and could be correlated with the degree of leptocytosis and/or hypochromia.

(*b*) *Mechanical fragility.*—Mechanical fragility of erythrocytes was measured by a modified method of Shen, Castle and Fleming (*Sceince*, **100,** p. 387, 1944). With this method the mean value in normal was 3·58 per cent and the range of variation was 2·32 to 4·8 per cent. In 15 cases of Cooley's anæmia the mean value was 5·18 per cent and the range of variation was 3·3 to 9·0 per cent. In 16 cases of Cooley's trait the mean value was 3·75 per cent and the range of variation was 1·9 to 6·5 per cent.

The findings suggest that in Cooley's anæmia the erythrocytes are more susceptible to mechanical trauma. Increased mechanical fragility explains to a certain extent the hæmolytic component which is a part and parcel of the disease. The paradoxical finding of increased erythrocytic resistance in hypotonic saline solution which is characteristic of the disease may be explained by the abnormally thin red cells which has the potential capacity of imbibing larger amounts of fluid. The dissociated behaviour of mechanical and osmotic fragility suggests that *in vivo* hæmolysis is mediated through lytic forces which are more mechanical than osmotic in nature.

Plasma hæmoglobin.—Plasma hæmoglobin was estimated in 90 subjects consisting of 25 normals, 22 patients with Cooley's anæmia, 13 cases of Cooley's trait and 30 cases of anæmia due to other causes. A modified method of Bing and Baker (*Jour. Biol. Chem.*, **92,** p. 589, 1931) was employed. Special care was taken to avoid hæmolysis during collection and manipulation of blood by (*i*) avoiding stasis, (*ii*) using wide bore needle, (*iii*) using syringe well rinsed with normal saline, and (*iv*) making clean venipuncture and ensuring ready flow of blood. The values in the differnt groups are shown in Table IV:—

TABLE IV.

Subject	Number	PLASMA HÆMOGLOBIN IN MG. PER 100 C.C.	
		Mean	Range
Normal	25	1·66	0·69 to 2·72
Cooley's anæmia ...	22	26·28	4·30 to 56·50
Cooley's trait ...	13	1·46	0·62 to 4·36
Anæmia due to other causes	30	1·18	0·21 to 4·35

Analysis of the data shows that plasma hæmoglobin level was significantly increased in Cooley's anæmia but not in Cooley's trait.

High level of plasma hæmoglobin in Cooley's anæmia suggests that intravascular hæmolysis is one of the factors in the causation of anæmia.

Fœtal hæmoglobin.—In 60 cases of Cooley's anæmia, fœtal hæmoglobin level, as measured by the alkali denaturation technique of Singer *et al.* (*Blood*, **6,** p. 413, 1951) ranged from 6·85 to 82·95 per cent with a mean value of 34·96 per cent. There was no significant correlation of fœtal hæmoglobin with the age of the patient, degree of anæmia, splenomegaly, reticulocytosis, normoblastosis, leptocytosis, the number of target cells in the peripheral circulation or fragility of the red cells, osmotic or mechanical. Degree of hæmolysis as indicated by plasma bilirubin and plasma hæmoglobin levels could not also be correlated with fœtal hæmoglobin.

(*a*) *Effect of blood transfusion.*—Transfusion of blood temporarily reduced the amount of circulating fœtal hæmoglobin, the degree of reduction being generally proportional to the amount of blood transfused.

Exchange transfusion was carried out in two patients through polythene tube introduced into the upper part of long saphenous vein near its junction with the femoral vein. In the first patient, an infant aged ten months and weighing 15 lb., 482 c.c. of patient's blood was replaced by 571 c.c. of donor's blood; at the end of transfusion fœtal hæmoglobin level dropped from pre-transfusion level of 16·06 per cent to 2·58 per cent. Thereafter the level remained low (within four per cent) for 30 days and then gradually rose to 10·7 per cent on the 65th day (Table V*a*).

TABLE V*a*.

Effect of exchange transfusion on fœtal hæmoglobin level in an infant aged ten months and weighing 15-*lb.*

	Date	Hæmoglobin, in g. per 100 c.c.	R.b.c., in millions	P.c.v., per cent	Fœtal hæmoglobin per cent
On admission	29–11–54	2·9	0·87	...	27·6
After 9 blood transfusions 50 c.c. each	2–3–54	4·93	1·89	...	16·06
Before exchange transfusion ...	23–4–55	4·06	1·29	15·0	...
At the end of exchange transfusion	27–4–55	9·86	...	30·0	2·58
24 hours after	28–4–55	9·86	...	...	...
72 ,, ,, ...	30–4–55	12·47	3·3	33·0	4·65
7 days ,, ...	4–5–55	11·31	3·15	33·0	3·48
10 ,, ,, ...	7–5–55	11·31	3·22	31·0	2·58
16 ,, ,, ...	13–5–55	8·99	2·55	28·0	2·62
23 ,, ,, ...	20–5–55	7·25	2·20	24·0	3·23
30 ,, ,, ...	27–5–55	7·54	2·27	...	2·16
37 ,, ,, ...	3–6–55	6·09	1·84	20·0	4·87
45 ,, ,, ...	10–6–55	5·22	1·81	17·0	5·29
51 ,, ,, ...	17–6–55	4·60	1·38	14·0	4·95
65 ,, ,, ...	1–7–55	3·19	0·97	10·0	9·37

In the second patient, aged two years and weighing 22 lb., exchange transfusion, which replaced 731 c.c. of patient's blood by 810 c.c. of donor's blood, reduced the fœtal hæmoglobin level from pre-transfusion level of 13·75 per cent to 4·92 per cent at the end of transfusion. On the sixth day following exchange transfusion the patient was splenectomized. Serial studies on blood values and fœtal hæmoglobin levels are shown in Table V*b*. During operation samples of blood collected from splenic artery and vein were examined for fœtal hæmoglobin content. Fœtal hæmoglobin content of splenic artery and vein were 5·96 and 5·63 per cent, respectively; foetal hæmoglobin in general circulation estimated two days previously was 6·41 per cent.

TABLE V*b*.

Effect of exchange transfusion and splenectomy in a child aged two years and weighing 22 lb.

	Date	Hæmoglobin, g./100 c.c.	R.b.c., in millions	P.c.v., per cent	Fœtal hæmoglobin, per cent
On admission	20–7–55	4·06	1·28	16·0	39·92
Before exchange transfusion ...	19–8–55	4·93	1·98	20·0	13·75
At the end of exchange transfusion	23–8–55	10·15	...	32·0	4·92
24 hours after exchange transfusion	24–8–55	12·76	3·27	35·0	5·32
48 hours after exchange transfusion	25–8–55	11·02	3·23	33·0	5·63
72 hours after exchange transfusion	26–8–55	10·73	2·98	33·0	6·31
96 ,, ,, ,, ,,	27–8–55	10·44	2·77	33·0	6·41
Splenectomy	29–8–55	...	...	...	...
10 days after exchange transfusion	2–9–55	9·57	2·8	34·0	8·33
14 ,, ,, ,, ,,	6–9–55	9·57	2·67	34·0	8·45
21 ,, ,, ,, ,,	13–9–55	11·02	3·05	...	9·04

(*b*) *Effect of therapy.*—Different hæmatinics including cobalt chloride even when continued for a long time did not show any change in fœtal hæmoglobin level.

(*c*) *Effect of splenectomy.*—Splenectomy done in four cases, including one in which a pre-operative exchange transfusion was given showed temporary depression of fœtal hæmoglobin level in the immediate post-operative period. This was probably due to blood transfusions received immediately before or during operation.

Serum-iron and iron-binding capacity of serum.—Mean serum iron and unsaturated iron-binding capacity of serum in 28 cases were 167·4 (range 75 to 335) and 37·1 (range 0 to 226) microgram per 100 c.c., respectively. High or normal serum-iron and low iron-binding capacity of serum were characteristic of the disease and proved useful in differentiating the disease from hypochromic anæmias of iron deficiency and infection.

Bone marrow.—Bone marrow was extremely hypercellular with normoblastic hyperplasia. Granulopoiesis and thrombopoiesis were relatively depressed. Hæmosiderin content of the bone marrow was increased.

Incidence of Rh(D).—Studies were undertaken to find out the incidence of Rh(D) in Cooley's anæmia in Bengali Hindus. Tests were carried out in cases of Cooley's anæmia and their parents and in normals and in other diseased states. Rh typing was done with anti-D serum received through the courtesy of Dr. A. E. Mourant of Lister Institute, London, using autoserum as diluent. Results are shown in Table VI:—

TABLE VI.

Incidence of Rh(D) in Bengali Hindus.

	Total number	Rh(D) + Ve		Rh(D) —Ve	
		Number	Per cent	Number	Per cent
Cooley's anæmia ...	43	34	79·1	9	20·9
Fathers of patients with Cooley's anæmia ...	22	19	86·4	3	13·6
Mothers of patients with Cooley's anæmia ...	23	20	86·06	3	13·04
Normal subjects or patients with other diseases ...	38	36	94·5	2	5·5

Comment.—In Indians incidence of Rh(D) negative individuals is lower than in Europeans or Americans. Whereas approximately 15 per cent of white population in Europe and America are Rh(D) negative, in Indians Rh(D) negative individuals vary from 2·5 to 10 per cent.

The present series though small show higher incidence of Rh(D) negative in Cooley's anæmia. While the incidence of Rh(D) negative individuals was as low as 5·5 per cent in general population, the incidence of Rh(D) negative in Cooley's anæmia belonging to the same racial population (Bengali Hindus) and in their fathers and mothers was distinctly higher being 20·9, 13·6 and 13·04 per cent, respectively.

Further work is in progress to find out if the higher incidence of Rh(D) negative in Cooley's anæmia has got any ætiological bearing.

Radiological changes in bones.—Skiagrams of skull, forearm and hand bones were taken in 22 patients with ages varying from three months to 22 years.

Skull bones.—Typical hair-on-end appearance was seen in only three cases. A generalized mottled appearance due to loss of density, increased porosity and coarse bony trabeculations were present in 16 cases. No definite change could be seen in the three younger patients aged three months, six months and two years.

Forearm and hand bones.—Characteristic stencilled striations giving rise to a mosaic pattern were seen in all the patients who had shown skull changes excepting one, a young adult aged 22 with massive splenomegaly and evidences of hypersplenism. Slight osteoporotic changes were also seen in the three younger patients who did not show any changes in skull bones.

Therapy.—Cooley's anæmia is refractory to all types of hematinics. There is some response to cobalt chloride which, however, is transitory. Studies were undertaken to evaluate the rôle of *splenectomy* in (*i*) cases with pronounced hæmolytic component as evidenced by high degree of reticulocytosis and hyperbilirubinæmia and marked splenomegaly, and (*ii*) in cases showing evidences of hypersplenism, where transfusion of blood failed to maintain the blood level for any appreciable period.

Splenectomy was done in five cases. Immediate response to splenectomy was slight improvement of blood picture with varying degree of clinical improvement. In all the cases, number of normoblasts and target cells in the peripheral blood increased to a considerable degree. The extent of normoblastosis before operation in these cases varied from 153 to 7,715 per cm.; after operation the normoblasts ranged from 1,892 to 1,056,681 per cm. Long term results in these cases are awaited.

III. COOLEY'S TRAIT.

Two hundred and two family members of patients suffering from Cooley's anæmia were investigated. One hundred and thirtyfour of the family members showed evidences of minimum anomaly or Cooley's trait.

On analysis of the data of family members, evidences of trait in the parents (either in one or in both) and/or the siblings could be traced in all the instances. Both the parents of 43 patients were available for study; evidences of trait were present in both the parents of 29 patients; evidences of trait in either parent was present in 11, while in the remaining 3, evidences of trait were equivocal.

One hundred and nineteen family members carrying the 'trait' and showing minimum anomaly were critically examined to find out their clinical, hæmatological and biochemical status. The results are given below:

Clinical.—All were usually asymptomatic. Splenomegaly, hepatomegaly and jaundice were uniformly absent.

Blood picture.—None had significant anæmia. Reticulocytes varied from 0·2 to 6·0 per cent with a mean of 1·34 per cent. Characteristic abnormality in red cell morphology of varying degree was present in all the cases. Occasional normoblast C was present in four cases and occasional neutrophilic myelocyte in two cases.

Target cells.—Number of target cells varied widely from 0·5 to 26·0 per cent. Mean target cell count was 2·04 per cent.

van den Bergh test.—Bilirubin content of plasma was within 0·6 mg. per cent in all the cases excepting one in which it was 0·8 mg. per cent.

Fragility of red cells: (a) Osmotic fragility.—Mean values of sodium chloride concentration indicating the beginning and completion of hæmolysis were 0·42 (range of variation 0·46 to 0·36) and 0·25 (range of variation 0·36 to 0·16) per cent, respectively. Osmotic resistance of red cells proved useful in identifying Cooley's trait.

(*b*) *Mechanical fragility.*—Mean value in 16 cases was 3·75 per cent and the range of variation was 1·9 to 6·5 per cent. Normal mean value with this method was 3·58 per cent and the range of variation was 2·32 to 4·8 per cent. The results indicate that red cells of patients with Cooley's trait do not show any abnormal susceptibility to mechanical trauma.

Plasma hæmoglobin.—Mean value of plasma hæmoglobin in 13 cases was 1·46 and the range of variation was 0·62 to 4·36 mg. per 100 c.c. of blood. In normals mean value of plasma hæmoglobin was 1·66 and the range of variation was 0·69 to 2·72 mg. per cent (Table IV). Normal plasma hæmoglobin values in Cooley's trait would suggest absence of abnormal intravascular hæmolysis.

Fœtal hæmoglobin.—Mean fœtal hæmoglobin level in 70 cases was 3·48 per cent and the range of variation was 0·71 to 4·39 per cent. The values were within normal range and significantly lower than those in Cooley's anæmia.

Serum-iron and iron-binding capacity of serum.—Mean serum iron in 51 cases was 78·1 and the range of variation was from 25·0 to 170·0 microgram per 100 c.c. Analysis of the results showed that in 11 cases the values were 50·0 or less and in others the values were above 50·0. Mean unsaturated iron-binding capacity of serum was 275·3 and the range of variation was from 175·0 to 467·0 microgram per 100 c.c.

The values of serum-iron were thus lower and those of iron-binding capacity of serum higher than the values in Cooley's anæmia. Except in 11 cases which showed low serum-iron level, the values were within normal range. The pattern of serum-iron level and iron-binding capacity of serum did not, therefore, provide any diagnostic clue.

Bone marrow.—Bone marrow studies were done in six cases. The relative proportion of normoblasts, granular and nongranular cells are shown in Table VII. Slight increase of normoblasts was seen in two cases (Nos. 1 and 2). There was no significant change in the granular cells and megakaryocytes.

TABLE VII.

Number	Normoblasts	Total granular cells	Non-granular cells
1	36·0	52·6	11·4
2	37·6	57·6	4·8
3	31·4	60·4	8·2
4	32·8	58·0	9·2
5	32·6	63·6	3·8
6	28·4	59·2	12·4

Radiological changes in bones.—Fourteen persons were examined radiologically for evidences of abnormal bony pattern. In 12 persons the pattern in the skull and in the long bones was different from normal and simulated to some extent the pattern seen in Cooley's anæmia.

IV. IRON STUDIES IN KALA-AZAR.

(*a*) *Serum-iron and iron-binding capacity of serum.*—Through courtesy of Dr. P. C. Sen Gupta, Officer-in-Charge, Kala-azar Research Department, School of Tropical Medicine, Calcutta, serum-iron (S.I.), and total iron-binding capacity of serum (T.I.B.C.) were determined in 17 cases of kala-azar before the institution of therapy (Table VIII). Mean S.I. was 36·0 microgram per 100 c.c. (range 5 to 70) and mean T.I.B.C. was 252·0 (range 100 to 340). The pattern indicated hypoferremia with depressed iron-binding capacity of serum. There was, however, no significant correlation between the degree of anæmia and the degree of hypoferremia. Studies were repeated in 12 cases after full regime of specific therapy which effected clinical cure and hæmatological improvement in all the cases excepting one (No. 12). In nine cases along with clinical and hæmatological improvement the mean values of S.I. and T.I.B.C. rose to 74·0 and 310·0, respectively. Of the remaining three cases (Nos. 6, 8 and 12) which did not show any significant alteration of S.I. and T.I.B.C. level, one (No. 12) was refractory to treatment.

(*b*) *Absorption pattern with ferrous sulphate.*—Mean maximum rise of serum iron following 0·4 g. of ferrous sulphate in ten patients was 21·8 (range 0 to 60) μg. per 100 c.c. Repeat studies in eight patients after therapy showed that absorption rate had increased considerably, the mean value of maximum rise in serum-iron level being 76·2 (range 10 to 285) μg. per 100 c.c.

(*c*) *Hæmosiderin content of bone marrow.*—Following a modified method of Rath and Finch (*Jour. Lab. Clin. Med.*, **33,** p. 81, 1948) hæmosiderin content of bone marrow was estimated in 13 patients before therapy. The hæmosiderin content was scanty in 7 (Nos. 1, 3, 5, 7, 8, 12 and 14), moderate in amount in 5 (Nos. 4, 11, 13, 15 and 17) and greatly increased in one. Repeat estimations in six cases after thearpy showed that the pattern was unchanged in 3 (Nos. 3, 5 and 8); the hæmosiderin became scantier in two cases (Nos. 13 and 15) and increased in one case (No. 11).

Conclusion.—Low serum iron, depressed iron-binding capacity of serum and poor absorption rates simulate the pattern in infection. Scanty hæmosiderin deposit in bone marrow indicates depletion of reserve iron stores. Works are in progress to elucidate the significance of these findings.

V. SERUM STUDIES IN LEPROSY.

Through courtesy of Dr. N. Mukherjee, Officer-in-Charge, Leprosy Department, School of Tropical Medicine, Calcutta, iron studies were carried out in 21 cases of leprosy consisting of neural type 14 and lepromatous type 7. All the patients had r.b.c. count above 3·0 millions.

In the neural type mean values of serum-iron and total iron-binding capacity of serum were 94·3 (range 50 to 205) and 361·3 (range 270 to 463) microgram per 100 c.c., respectively. In the lepromatous type the mean values for serum-iron and total iron-binding capacity of serum were 79·3 (range 70 to 125) and 344·0 (range 280 to 393) per 100 c.c., respectively.

The values for both serum-iron and iron-binding capacity were within normal range.

TABLE

Iron studies

Number	Name	Sex	SERUM IRON/μg. PER CENT		TOTAL IRON BINDING CAPACITY OF SERUM μg. PER CENT		HB. IN G./100 C.C.		R.B.C. in MILLION	
			Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1.	P. P.	F	40	70	240	20	9·52	11·89	3·32	3·6
2.	J. C. D.	M	70	75	270	348	8·41	12·18	2·43	3·5
3.	M. A.	M	45	50	290	410	9·28	11·02	2·93	3·31
4.	Md. I.	M	50	120	220	353	5·8	12·47	1·62	3·49
5.	A. S.	F	50	60	100	260	9·28	8·99	2·9	3·03
6.	A. B.	F	45	45	315	316	7·54	10·73	2·56	3·8
7.	S. C. B.	M	5	40	110	340	6·09	8·41	1·88	3·01
8.	L. S.	M	45	30	278	263	7·54	11·6	2·39	3·35
9.	R. D.	F	15	...	215	...	8·7	10·8	2·7	3·95
10.	U. T. D.	F	25	...	165	...	8·12	10·2	2·57	2·87
11.	N. B.	M	25	...	225	...	8·99	9·28	2·76	3·41
12.	B.	M	10	15	146	115	4·35	5·1	1·59	2·23
13.	A. B.	M	40	70	240	270	6·96	11·6	1·74	3·65
14.	S.	M	5	30	205	370	8·41	...	2·56	...
15.	S. B.	F	50	100	270	350	10·73	10·73	2·91	3·62
16.	T. D.	M	50	...	250	...	0·38	...	1·86	...
17.	R. K. D.	M	40	...	340	...	6·96	14·28	2·03	4·10

VIII.

in kala-azar.

MAXIMUM RISE IN SERUM IRON WITH 0·4 G. OF FERROUS SULPHATE		Treatment in addition to specific treatment for kala-azar	HÆMOSIDERIN CONTECT OF BONE MARROW		Remarks
Before treatment	After treatment		Before treatment	After treatment	
10 in 2 hrs.	10 in 6 hrs.	*Nil*	Scanty	...	Cured of kala-azar
10 in 6 hrs.	60 in 6 hrs.	,,	...	...	,,
30 in 6 hrs.	285 in 6 hrs.	,,	Scanty	Scanty	,,
50 in 6 hrs.	45 in 6 hrs.	,,	+	...	,,
60 in 6 hrs.	90 in 6 hrs.	,,	Scanty	Scanty	,,
...	...	,,	...	...	,,
...	...	,,	Scanty	...	,,
...	...	,,	Scanty	Scanty	,,
...	...	Ferrous sulphate	...	...	,,
45 in 6 hrs.	...	*Nil*	...	...	,,
...	...	Had ferrous sulphate and liver extract previously	+	++	,,
0 in 6 hrs.	15 in 2 hrs.	*Nil*	Scanty	...	Resistant kala-azar
0 in 6 hrs.	30 in 2 hrs.	,,	+	Scanty	Cured of kala-azar
...	75 in 6 hrs.	,,	Scanty	...	,,
...	...	,,	+	Scanty	,,
13 in 2 hrs.	...	,,	+++	...	Died
0 in 2 hrs.	...	Ferrous sulphate and liver extract orally	+	...	Cured of kala-azar

5. Clinical Research Unit under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

SPLENOMEGALY.

One hundred and thirty-four cases of chronic idiopathic splenomegaly including those of the last two years were investigated as indoor patients and followed up for varying periods and the data, recorded in a special proforma, analysed.

(1) *Clinical picture.*—Ninety-four patients (70 per cent) were young people between 10 and 30 years of age. There were 114 males and 20 females. Vast majority of them came from the malarious rural areas of Bengal, some from Orissa and a few from Bihar. Their economic condition was generally poor and in most of them there was evidence of malnutrition. The onset was insidious and the duration was up to ten years in this series. History of fever from time to time was common and many of them were treated as malaria or kala-azar in the past. The fever was low and intermittent in most cases and irregularly remittent in some. But there was a small group of 12 patients whose primary complaint was recurrent attacks of hæmatemesis and/or melæna, and fever was not a feature except before or during the bleeding episodes. In two patients this bleeding episode recurred at regular intervals and they could even foretell the probable time of an attack. Seventy-six patients (56 per cent) had splenic enlargement between five and ten inches below the tip of the left ninth costal cartilage. The spleen was firm or hard, painless and more or less mobile. Size of the spleen was at times noticed to be more enlarged following a febrile attack, somewhat receding again as the fever subsided. It was invariably diminished after hæmatemesis or melæna. The liver was enlarged up to 3½ inches below the costal margin except in six cases in which it was not palpable. Enlargement of the left lobe was greater than of the right in a large proportion of cases. It was usually firm, mostly smooth but in some cases the surface was irregular. Anæmia moderate or severe was an associated feature in 93 cases (70 per cent). Seventeen patients were admitted with jaundice of varying intensity and twenty-four with ascites with or without œdema of the legs. Bleeding from the gums and sometimes from the nose was frequently complained of.

(2) *Laboratory findings:* (*a*) *Blood.*—The anæmia was usually orthochromic and slightly macrocytic in type. Leucopenia, which was almost invariably present along with anæmia, mainly affected the neutrophils. Pancytopenia was observed in several cases including marked diminution of r.b.c., w.b.c., and platelets. The sedimentation rate of red blood cells was very much increased. No abnormal blood cells were seen.

Malaria parasites were found only in two cases (*vivax* and *quartan*). Microfilaria was present in the night blood of three patients and kala-azar was, of course, excluded in every case. W. R. was positive in 19 cases. Slight increase in bleeding time was common but coagulation time was usually within normal limits. Fragility of r.b.c. was normal.

(*b*) *Bone marrow.*—Bone marrow was studied in twenty-eight anæmia cases. The marrow was hypercellular with normoblastic erythropoiesis in all, and iron deficiency normoblasts were present in 50 per cent cases. Granulopoiesis was relatively depressed with regard to neutrophils but there was no change in so far as the eosinophils were concerned except in four cases in which it was increased. There was no significant change in lymphopoiesis. Megakaryocytes were mostly active and adequate in number. No parasites were found.

(*c*) *Hepatic function.*—This included determination of serum proteins, thymol turbidity, serum-bilirubin content, prothrombin time and in some cases excretion of urobilinogen in the urine. The total serum proteins were within normal limits, but the ratio of albumin and globulin was altered and the ratio was reversed in 62 cases examined. The thymol turbidity test, done in 88 cases, showed high values in 63. Serum-bilirubin content was above 1 mg. per cent in 22 cases. Prothrombin time was also slightly raised in the majority of the cases. Excretion of urobilinogen in the urine was usually high. The above tests indicate that the liver of many of them was not functioning normally.

(*d*) *Hepatic biopsy.*—Liver biopsy specimens were examined in 45 cases (needle biopsy material in 33 cases and specimen obtained during operation in 12). The histological picture appeared normal in 14 cases, while the rest showed varied changes. Degenerative changes of hepatic parenchyma, focal or diffuse, with infiltration of lymphocytes, fibroblasts and occasional histiocytes and plasma cells were prominent features. Fatty changes were observed in seven cases. On reticulin stain there was condensation of reticulin fibres at the degenerated areas. Nearly half of them showed evidence of portal fibrosis with infiltration of mononuclear cells, and even extension of the young proliferating connective tissues into the lobules. These changes were more of the nature of replacement fibrosis. The liver sinusoids were found to contain clumps of round cells in many cases. Prof. P. N. Wahi, Agra, kindly examined six liver biopsy specimens and noticed degeneration of liver cells in all and portal fibrosis in four. The most significant change observed by him was a severe degree of reticulo-endotheliosis in most cases, and assumed the clumps of round cells in the sinusoids as Kuffer cells with hyperchromatic nuclei and containing brownish pigment. Reviewing these and some of the old slides he was of the opinion that the most of these cases of so called 'tropical splenomegaly' show changes compatible with those in chronic malaria and the most prominent change is the activity of reticulo-endothelial cells.

(*e*) *Spleen.*—The enormous size of the spleen associated with anæmia and leucopenia encountered in these cases, suggested a state of hypersplenism (i.e., an overactive spleen in which the blood was sequestrated leading to excessive destruction of r.b.c., granulocytes and platelets to a varying extent). A fair idea could be obtained by injecting a small dose of adrenaline (½ c.c. of 1:1,000) and noting the hæmatological response at short intervals. The maximum response occurred after 10 to 30 minutes and usually coincided with the maximum splenic contraction which varied from 1″ to 4″. There was definite rise of r.b.c. count and cell volume above the initial level, the mean being 1,170,000 per c.mm. and 7·8 per cent, respectively, whereas the leucocytic rise was variable. The marked increase in erythrocytes coinciding with the splenic contraction indicated their stagnation in large numbers in the spleen. The sequestration phagocytosis of blood cells by the spleen leads ultimately to their depletion in large number, though at first compensated by the hyperplasia of bone marrow. Some response to adrenaline may occur in normal subjects, but never to the same extent as seen in these patients with splenomegaly. The test was also done in a few splenectomized cases which showed only negligible rise of r.b.c. count. The improvement following hæmatinics was usually not maintained long apparently due to hypersplenism.

The spleen when removed by operation was found to be considerably enlarged weighing from two to six lbs. The capsule was thickened with evidence of adhesions. The cut surface was reddish brown, firm with prominent trabeculæ. Normal histological appearance of the spleen was completely lost. The capsule was thickened and the trabeculæ were in most cases markedly prominent. There was great increase of reticulin fibres. The sinuses were congested and dilated to variable extent. In the congested sinuses, there were erythrocytes, lymphocytes, a fair number of neutrophils, a few eosinophils, some monocytic histiocytes, as well as giant cells. Erythrophagocytosis by the mono and mutli-nucleated giant cells was evident in a fair number of cases. The red pulp of the spleen showed definite proliferation of the pleomorphic cells of the reticulo-endothelial system including giant cells. The malpighian follicles were atrophied and in one preparation it was almost absent. Areas of infarction were noticed in two cases. In one case there were large number of histiocytes containing hæmosiderin granules round the zone of infarction. There was no evidence of malarial infection even in the cases that had malarial attacks after splenectomy.

(3) *Radiological findings:*

(*a*) *Splenoportal venography.*—Altogether 55 cases have been subjected to splenoportography after a precutaneous injection of a radio-opaque substance (38·5 per cent iodoxyl) into the spleen. Nothing particular was seen in the spleen except local concentration of the dye and in some cases visualization of the splenic venules which appeared dilated. Splashing of the dye between the capsule and splenic substance was noticed in three cases. From the spleen the dye could be traced through the splenic and portal veins to the liver. A common abnormality was marked dilatation and tortuosity of splenic and portal veins. This dilatation and tortuosity appeared reciprocal to the enlargement of spleen. Loop formation of splenic vein was also noticed in one case. Four cases showed anomalous variation of the venous channels; in one case the portal vein was not at all visualized and the splenic vein was narrow and terminated abruptly and in two cases, the course of the splenic and portal veins appeared abnormal. Cavernomatous transformation of the portal vein was noticed in one. Part of the splenic vein appeared narrowed in two cases suggesting thrombosis and recanalization. Visualization of the collaterals, e.g., short gastric, left gastric, pancreatico-duodenal and gastro-duodenal was noticed in 18 cases indicating the passage of the dye against the usual direction of flow. It may be mentioned here that cases showing radiologically demonstrable œsophageal varix usually showed collateral veins but the reverse was not true. The maximum diameter of the splenic vein in well visualized cases varied from 6 mm. to 30 mm. and that of the portal vein from 11 mm. to 35 mm. as reported last year. The portal radicles were visualized inside the right lobe of the liver in all except nine cases including the two cases showing abrupt ending of portal vein, and in the left lobe in two only. Incidentally, it may be mentioned that biliary calculi were seen in four cases.

(*b*) *Barium meal examination.*—Out of twelve patients admitted with symptoms suggestive of portal hypertension, e.g., hæmatemesis and/or melæna with enlarged spleen, œsophageal varices were demonstrated in ten and absent in other two even on œsophagoscopic examination. Of the remaining patients having fever and splenic enlargement as their main complaints, only one showed presence of œsophageal varix. This case was reported to have hæmatemesis later on. Varicose condition of the greater curvature of the stomach was demonstrated in one besides œsophageal varix. The stomach in all cases showed a dextro-deviation.

(*c*) *Pyelography.*—Intravenous pyelography done in 15 cases showed dropping of the left kideny, enlargement in size in the longitudinal diameter and poor concentration by the calyces and also clubbing in addition. Pyelography repeated after operation in three cases showed ascent of the kidney with good concentration of the dye.

(4) *Treatment:*

(*a*) *Medical.*—Nearly half of them improved in general health with diet alone. Hæmatinics, e.g., liver extract (crude) 3 c.c. intramuscular daily for six consecutive days and later on twice or thrice a week for a variable period and iron in the form of ferrous sulphate 15 gr. to 18 gr. a day for three to four weeks, were given to 75 patients with moderate or marked anæmia. There was improvement of anæmia in most of them after a single course, but a few cases failed to maintain the improvement and required a second course. In 34 cases with relatively short history (six months to one year) and spleen enlarged up to four inches, the fever was controlled with anti-malarials and the spleen diminished in size, to a variable extent, but the majority of cases with huge spleen up to ten inches, did not improve. Ascoli's treatment consisting in the daily intravenous administration of gradually increasing small doses of adrenaline, e.g., 1/100th, 2/100th, 3/100th mg., etc., up to 1/10th mg., which is then repeated making a total of 20 to 25 injections along with a mixture containing iron, quinine, arsenic and magnesium sulphate was tried in some cases without any remarkable effect. Cortisone was also tried in six cases empirically in daily doses of 50 mg. to 100 mg. In only two cases there was moderate reduction in the size of the spleen, but in others the results were not at all satisfactory.

Results.—As the patients came mainly from the distant villages of Bengal, Bihar and Orissa, regular follow-up was not possible. Only twenty one of them could be periodically followed up in the out-patient's department or by re-admission. The results are presented under following heads:—

(*i*) *General health.*—Though nearly half the patients improved in general health and gained weight with diet alone, they could not maintain it as their poor economic conditions did not permit. On re-admission in the hospital and with proper diet, marked re-improvement was noticed. Two patients did not show any improvement from the beginning, in spite of proper diet and other treatments, as they were in the late stage with clinical evidence of hepatic cirrhosis.

(*ii*) *Temperature.*—In some cases temperature subsided with anti-malarial combined with proper diet and hæmatinics, but recurred again after variable intervals.

(*iii*) *Blood picture.*—The hæmoglobin and r.b.c. count usually improved after treatment though not sustained, and on follow-up studies was found to deteriorate again, apparently due to hypersplenism.

(*iv*) *Spleen.*—The spleen was reduced in size with anti-malarials in the earlier stage of the disease with moderate enlargement, and on follow-up studies it was found to have enlarged again in some of them due to attacks of fever. In the later stage when marked fibrosis takes place, it did not respond at all.

(*v*) *Liver.*—Effect on the size of the liver was variable and could not be properly assessed. Follow-up liver biopsy study was not possible.

(*vi*) *Death.*—Three cases died in two years.

(*b*) *Surgical.*—As the gain in weight was not steady, and the initial low values of red blood cells, white cells and hæmoglobin showed little or no tendency to sustained improvement in spite of diet and hæmatinics, removal of the spleen was considered with a view to remove the factor of hypersplenism. Splenectomy was done in twenty-two cases with the addition of lieno-renal anastomosis in two and portacaval in one. Portal pressure taken during operation in ten cases at the gastrœpiploic vein was found high in two cases who gave history of hæmatemesis. In others it was found normal or only slightly increased. Liver appeared cirrhotic in four cases.

Results.—Four patients died during operation and one three days after from severe hæmatemesis. Another patient died one year after from cirrhosis of liver with ascites, œdema of legs, jaundice and hæmatemesis.

Of the 17 patients that survived after operation, 12 have been followed up for a period of one and a half to two years and the remaining five for six months to one year. Their progress is dealt with under the following heads:—

(*i*) *General health.*—Fourteen patients gained weight by 2 lb. to 16 lb. with the improvement of general condition including the one who died a year after and three others lost weight by 3 lb. to 7 lbs. after operation but after one year gradual deterioration of health with loss of weight was evidenced in nine of them due to one or other complication mentioned below:—

(*ii*) *Temperature.*—Six patients had malarial attacks due to *P. vivax* infection within a period of 14 days to 3 months after splenectomy. The number of attacks varied from one to four. These patients had malaria while still in the hospital and re-infection was most unlikely, and so also the possibility of infection through blood transfusion. One of them who had four attacks of malarial fever, developed cirrhosis of liver and finally died of hepatic coma. Four patients have been doing well, including the three who had shunt operation. Of the remaining seven patients, two have developed ascites apparently due to cirrhosis of liver complicated with right sided pleural effusion in one. The other five patients are suffering from irregular temperature as before operation (? hepatic fever).

(*iii*) *Hæmatemesis and melæna.*—Three patients had hæmatemesis and melæna before operation and both of them had lieno-renal anastomosis in addition to splenectomy. One of them who complained of bleeding episode every five months, had severe hæmatemesis on the third day of operation near about the completion of five months and it is strange to note that again after five months he had another attack of melæna though no hæmatemesis. Barium-meal examination done about a week before the bleeding showed presence of œsophageal varix. Another patient who was admitted with history of fever simulating malaria, had hæmatemesis one year after operation and finally died of hepatic coma.

(*iv*) *Jaundice.*—Five patients developed jaundice within three to twelve months after operation. Two of them had it along with malarial paroxysms.

(*v*) *Blood picture.*—The blood picture improved to a great extent within one month after operation and this improvement is more or less being maintained in all the survivors. Hæmoglobin, r.b.c. and leucocyte count increased from an average of 10·0 mg. per cent, 2·8 millions and 4,400/c.mm. before operation to 13·5 g. per cent, 4·1 millions and 12,500/c.mm. after one month to 14·4 g. per cent, 4·2 millions and 12,400/c.mm. after six months and to 14·7 g. per cent, 4·25 millions and 11,800/c.mm. after one year of operation, respectively.

The increase in leucocytic level was associated with moderate or high eosinophilia up to a maximum of 53 per cent (10,300/per c.mm.) and this was temporarily lowered by any febrile attack including malaria probably due to increased secretion of cortical hormones. The eosinophilia was, however, found reduced towards the end of the year.

(*vi*) *Bone marrow.*—In six cases the bone marrow was studied both before and after operation. The marrow was normocellular and normoblastic with relative depression of neutrophil granulopoiesis in only one case against five before operation and increase of eosinophil in all except one.

(*vii*) *Liver.*—Against the improvement that took place in the clinical condition in many cases and blood picture in all after operation, its effects on the liver were of interest. As a rule, liver in all cases increased by ½" to 1" after operation, but gradually came down and in three cases it has become very much reduced in size and developed into clinical picture of cirrhosis with ascites and jaundice. Biopsy done in six patients within a variable period of three to six months after operation showed the same degenerative changes of hepatic parenchyma as seen before it and in addition fatty changes in two cases which were not seen previously. Other findings were moderate condensation of reticulin fibres at the degenerated areas as seen before operation and in general larger accumulation of lymphocytes in the sinusoids with hyperplasia of Kupffer cells. It was not possible to do the biopsy in the case in which the shunt operation was done.

Comment.—From a study of the data mentioned above, the cases can be broadly divided into three groups. In the first group (34 cases) the patients appeared to be cases of chronic malaria and malnutrition. There was little or no evidence of hypersplenism and the liver changes were seen only in three out of twelve cases in which biopsy material was examined. Medical treatment was of value in these cases, and when combined with better food, they could resolve to a normal or almost normal state. This is also borne out by our observation of similar cases in rural areas, where we had carried out chemoprophylactic treatment against malaria for five years.

The second group which included majority of the cases (88 in this series) had well marked signs of hypersplenism. It seems to be an advanced stage of the first group, but anti-malarial treatment had no effect on the spleen and liver, while treatment with hæmatinics though causing temporary improvement of anæmia, was followed by relapses. Liver showed pathological changes in 28 out of 33 cases in which biopsy material was studied.

The pathogenesis of this group of splenomegalies is probably as follows: In certain individuals recurrent attacks of malaria, in the absence of antimalarial therapy and proper diet leads to the enlargement of the spleen and liver. The splenic enlargement is evidence of imperfect host parasite adjustment. The defense mechanism of the body works to the utmost, leading to hyperplasia of the reticulo-endothelial cells, so that the spleen being the largest hoarder of this tissue undergoes considerable enlargement. There is concommitant development of the fibrous tissue as a supportive frame-work. Latent malaria stimulates reticuloendothelial hyperplasia, while hæmosiderin pigment stimulates fibrosis. The contractile power of the spleen is thereby diminished, especially in the advanced stage and blood is held back in the dilated sinuses, thus exposing the blood cells to greater phagocytosis than in the case normally. This hypersplenism—stagnation and destruction of large amount of blood in the spleen, accounted for the persistent anæmia in our cases, as evidenced by the adrenalin test, recurrence of anæmia after discontinuance of hæmatinics and examination of the spleen after operation. The bone marrow is stimulated to form more blood cells and becomes hypercellular. The liver shows evidence of parenchymatous degeneration with accumulation of round cells. This degeneration being progressive there is condensation of the reticulin fibres which may later be replaced by fibrous tissue. These changes in the liver and spleen may in course of time lead to portal obstruction and hypertension.

Lastly, there was a small minority group (12 in this series) in which the primary feature was the frequent gastro-intestinal hæmorrhage (hæmatemesis and/or melæna). Vascular changes, such as œsophageal varices, dilated collateral vessels, and thrombosis of portal or splenic vein have been observed by venography and/or during operation. Eight of them belonged to well-to-do family and had no history of malaria in the past. Liver biopsy studied in three cases only showed pathological changes in all. Adrenalin test done in two cases showed some evidence of hypersplenism in one.

Twenty-two patients (17 belonging to the second and five to the last group) were subjected to splenectomy with shunt operation in three. The portal pressure was measured during operation in eight cases of group II and two of group III. In the former it was normal (in majority) or slightly raised, while in the latter it was very high.

From a study of these operation cases it appears that pre-existing pathological changes in the liver determine the prognosis after splenectomy. Before deciding on the operation a biopsy study of the liver material should be made. Significant liver changes should be considered as a contra-indication to the operation.

(5) *Experimental studies.*—The cellophane method adopted last year has been utilized on a larger series of animals.

Eighteen albino rats weighing from 150 g. to 175 g. were used for this purpose. Under ether anæsthesia the abdomen was shaved and opened with aseptic precautions. The size of the spleen was measured both in longitudinal and transverse directions. A piece of cellophane paper was wrapped round a segment (0·3 cm. to 0·5 cm.) of the portal vein. The abdomen was closed in one layer of stitch. Penicillin 10,000 units b.d. was given for three days.

After three months the abdomen was re-opened under ether anæsthesia. The spleen was found to be markedly enlarged (more than three or four times) in six cases. There was, however, little or no enlargement in others. In these cases, the omentum, lower pole or hilum of the spleen and other intra-abdominal structures were found adherent to the abdominal wall; vascular communications between them (portal and systemic circulations) were distinctly seen which might possibly be a factor in combating the rise of portal tension and splenic enlargement. Of the six cases showing splenic enlargement, two showed marked dilatation and tortuosity of splenic vein and it could be traced to the point of occlusion. Tributaries of the splenic vein were also found to be dilated in three cases. Collection of clear straw-coloured fluid was found in the peritoneal cavity of three rats. Splenoportal venography by injection of a radio-opaque dye (38·5 iodoxyl) into the spleen was done in three cases out of the six showing enlarged spleen. All of them showed complete occlusion of portal vein at the point of cellophane wrapping with or without dilatation of the splenic vein and visualization of collaterals. In contrast, venographies of normal rats showed the whole extent of portal vein with its ramifications in the liver. Histological examination of the spleen showed thickened capsule and trabecultæ, dilated and congested sinuses and proliferated reticulo-endothelial cells in some cases. The liver did not show any gross abnormality.

This observation shows that occlusion of portal vein and consequent portal hypertension with some of its important manifestations, viz., splenomegaly, formation of collaterals and ascites can be produced experimentally in at least 33 per cent of the rats with cellophane paper method.

In collaboration with Dr. A. K. Basu seven operations have been performed on animals. In three cases, the splenic vein was primarily ligated in order to study its effect on spleen. In two cases artificial arteriovenous shunt between the splenic artery and vein was attempted but due to small size of the vessels, the particular operation was not successful and the operation terminated with ligation of the splenic vein and artery. In the remaining cases arterial anastomosis was done.

The animals all lived after the operation. It is now about three months since these operations were performed and animals will now be sacrificed and their spleens studied.

EPIDEMIC DROPSY.

Twenty-four albino rats, weighing from 40 g. to 60 g., were divided into four equal groups. First and second groups were injected with the total alkaloids of argemone intraperitoneally in the dose of 1·5 g./100 g. body-weight daily up to 15 injections. Third and fourth groups had similar doses subcutaneously. Along with the injections of argemone alkaloid the animals of the second and fourth groups were injected with hydrocortisone in the dose of 10 mg./kg. of body-weight. The results are presented in the Table I.

TABLE I.

Group number	Route of administration	Drug	Ascites	DEATH	
				Following ascites	Without ascites
1	Intraperitoneal	6 *Nil* (control)	4	1	0
2	”	6 Hydrocortisone	2	1	3
3	Subcutaneous	6 *Nil* (control)	2	0	2
4	”	6 Hydrocortisone	0	0	4

Thus, it appears that ascites were more frequent after intraperitoneal than with subcutaneous administration, and there was more death and less ascites formation in the drug-treated group than the control one.

The experiment was repeated with subcutaneous injection only (as the peritoneal irritation might be a factor in the causation of ascites with intraperitoneal injection) and with smaller doses of hydrocortisone.

Twelve albino rats, weighing from 40 g. to 70 g. were injected with argemone alkaloid subcutaneously in doses of 1·5 to 2 mg./100 g. of body-weight daily up to 15 injections. Six of them had injections of hydrocortisone in the dose of 2·5 mg./kg. of body-weight simultaneously. Of the six rats treated only with argemone alkaloid, four died with ascites in two. But none of the animals treated with hydrocortisone and argemone alkaloid simultaneously, developed ascites or died.

Comments.

Hydrocortisone appears to have some protective action when used in a dose of 2·5 mg./kg. of body-weight, against chronic argemone poisoning. with special reference to ascites formation.

THERAPEUTIC TRIALS.

(1) *Antimalarials:*

(*a*) *Amrit Sagar pills.*—(Received through the Secretary, Indian Council of Medical Research). This proprietary preparation prepared at the Ayurvedic Medical Hall, Lucknow, each pill containing about 2 gr. of quinine hydrochloride along with some indigenous drugs, was tried in six cases of acute malaria, 5 *vivax* and 1 *falciparum*, the usual dosage for an adult being three pills a day (at hourly intervals) for two consecutive days, followed by one pill twice daily for 14 days. One case was not given the follow-up dosage with a view to observe the immediate effect, and another, a child of five years had a quarter of the adult dose. The immediate effect of the preparation was good, the fever subsiding within 48 to 72 hours and the asexual parasites disappearing in 24 to 48 hours. The *vivax* sexual parasites were also cleared quickly. Crescents were unaffected. Three patients (including the one who did not have the follow-up regime) relapsed within two to four weeks while still in the hospital and were treated with other anti-malarials. On discharge from the hospital one patient had a relapse after six weeks and the other two could not be followed up. Though tried in a small series of cases, no synergism was evident in this clinical study. No untoward reactions were evident.

(*b*) *Supazine.*—(M/s. Bengal Immunity Co. Ltd.) A new synthetic anti-malarial, the chemical composition being 2:4 diamino-l-p-sulphonamidophenyl-1:6-dihydro-6:6 dimethyl-1:3-triazine hydrochloride was tried in 11 cases of acute malaria, eight *vivax* and three *falciparum*. The adults were given in the dosage of one tablet (0·26 g.) six hourly for four days. A child aged three years was given ½ tablet, twice daily, for four days and two others aged seven and eight years were given one tablet twice daily for four days. Fever subsided in four days in all except one *falciparum* case, where the temperature continued up to the sixth day. Asexual parasites disappeared from the peripheral blood in two to five days, while the gametocytes were practically unaffected. No untoward symptoms were encountered. There was recurrence of fever with asexual parasitæmia in four patients, while still in the hospital, three *vivax* and one *falciparum*, 8, 16, 17 and 9 days, respectively, after the temperature was normal. Two *vivax* cases were given one tablet weekly for three weeks as suppressive dosage. One of them relapsed, while in the third week of suppressive treatment and the other is being followed up for two weeks after the suppressive treatment has been completed.

(*c*) *Single dose treatment with camoquin primaquine.*—Fifteen cases of acute malaria, eight *vivax*, six *falciparum* and one *malariæ* were treated with a single dose of camoquin (0·45 g.) and primaquine (30 mg.). The fever subsided and the asexual parasites disappeared from the peripheral blood within two days in fourteen cases. In the remaining one, fever subsided within three days and so also the asexual parasites. None of the cases had relapsed within three to four weeks of their hospital stay.

(*d*) *Chemoprophylaxis with camoquin primaquine.*—Part of the study with camoquin and primaquine in fortnightly doses as a prophylaxis against malaria, carried out on 200 villagers (another 200 being kept as control) was reported last year. This trial could be continued till the middle of October, 1954 and the effect of prophylactic regime on the parasite rate per 100 is shown in Table II:—

TABLE II.

Group	June/July	August	September
Combined camoquin and primaquine ...	3	0	0
Control ...	4·1	3·8	8·9

The absence of any parasite positive case in the treated group in comparison to the rise from 4·1 to 8·9 appears to be significant. The incidence of overt malaria could not be recorded as it was not possible to examine the blood during febrile attacks. On enquiry at fortnightly visits, however, it was gathered that the incidence of fever was very insignificant in the treated group.

Comment.—Fortnightly doses of combined camoquin (160 mg.) and primaquine (10 mg.) have an effective suppressive action against malaria.

(2) *Antacid:*

(*a*) *Nulacin.*—(Received through the Secretary, Indian Council of Medical Research). Nulacin prepared by Horlics Limited was tried in a series of radiologically proved duodenal ulcer with hyperchlorhydria. Each tablet contains whole milk combined with dextrins and maltose and incorporating—Mag. trisilicate 3·5 gr., Mag. oxide pond. 2 gr., calcium carbonate 2 gr., Mag. carb. pond. 0·5 gr., oil menth. pip. q.s. and yields about 11 calories. During hospital treatment the average dose was one tablet to be placed in the mouth and slowly dissolved (taking half hour) four-hourly.

Pain subsided after two to three days treatment but they also had modified sippy diet along with it. In the follow-up treatment it was found that the patients liked these handy palatable tablets.

(*b*) *Gastric analysis with nulacin.*—In three patients with hyperchlorhydria gastric analysis was repeated after the administration of nulacin tablets, in two cases one tablet each and in another two tablets given at an hour's interval. Three samples were drawn in each case when one tablet was given, the free acid dropped by 35 units in one and to zero level in another but the curve rose again after one hour, almost to hyperchlorhydric level. In the case where two tablets were given, free acid dropped to zero after the first tablet, remained for one hour at the level and after the second tablet free acid remained in zero level for 45 minutes longer and then rising to hyperchlorhydric level. It appears that in this dosage nulacin is short acting antacid.

(3) *Amœbicide:*

(*a*) *Intes-steril.*—(Received through the Ministry of Health, Government of India). Intes-steril (Dr. Wild & Co.) contains neomycin sulphate 166 mg. per tablet (adult dosage) along with phthalyl sulphathiazole, methyl paraoxybenzoate and propyl paraoxy benzoate. Indications for its use are varied including amœbic dysentery. It was tried in a small series of acute amœbic dysentery in doses of one tablet every four hours for six days and found to be ineffective.

(*b*) *Camoform.*—(Received through the Secretary, Indian Council of Medical Research). A new anti-amœbic compound P.A.A. 70 (P.D.) containing neither arsenic nor bismuth in its molecule (diallyl-diethylaminœthylphenol dihydrochloride) and structurally related to amodiaquin has been subjected to clinical trial. So far it has been used only in ten cases; eight having active dysenteric symptoms with passage of mucus and blood and *E. histolytica* in the stool, one with recent attack of dysentery and E. *histolytica* trophozoites and cysts in the stool and another with history of alternate constipation and diarrhœa and *E. histolytica* cysts only in the stool. The drug was used in the daily dosage of 1 g. for seven to ten days in adults and smaller doses in children. In four cases there was good response, the stool being cleared of parasites in two to six days, while the remaining six patients failed to respond. The course was repeated in three cases with larger doses (1·5 g. daily for ten days in adults) with varying results. It has no effect on hepatitis which was present in one case. All the six cases were subsequently treated with emetine and other amœbicidal drugs and discharged apparently cured. No toxic effect was observed.

6. Liver Diseases Research Unit under Dr. P. N. Wahi at the S. N. Medical College, Agra.

SUMMARY.

1. An experimental study was undertaken to assess the rôle of adrenal cortex in the reversal phenomenon of cirrhosis induced by carbon tetrachloride in rats. It has been observed that the reversible stage of cirrhosis is associated with a normally functioning adrenal or one which can at least be induced to further activity when subjected to further stress. With the onset of irreversible cirrhosis, such property of the adrenal seems to be permanently damaged.

2. As a corollary to the above experiments, a batch of rats which had acquired cirrhosis of the liver was put on cortisone therapy. The severity of cirrhosis in these animals was compared with that of controls which were not given such injections. It was noted that cortisone helped regress the fibrosis when the cirrhosis was in the reversible stage. Once irreversible cirrhosis was, however, established, cortisone failed to undo it.

3. It had been observed by us in last year's Report that carbon tetrachloride induces a rise of free amino-acid content of the liver in the prenecrotic phases, after a single injection in rats. It was observed by Patwardhan *et al.* (*Ind. Jour. Med. Sc.*, **7,** p. 533, 1953) that there is, in addition, also a fall in the glutathione levels. Since these biochemical changes can also be induced in states of alarm reaction, a study was undertaken to investigate to what extent the adrenal cortex was responsible for these observations. It was observed that the peak of adrenal-cortical activity did not coincide with the peak of early biochemical changes observed in the livers, thus adding another evidence to our postulate (last year's Report) that the action of the chemical on the hepatic cells is a direct one.

4. The mode of direct action of carbon tetrachloride on the hepatic cell in acute injury in inducing certain enzyme changes in the rat was studied. As a result, it is felt that the action is metabolic in nature rather than chemical.

5. A study to investigate the rôle of necrotic tissue in the central 1/3rd of the lobule in inducing the retention of alkaline phosphatase in rat liver after a single injection of carbon tetrachloride was undertaken. It is felt that the necrotic tissue plays no part in the induction of the above mentioned change.

6. Thirty-six cases of congestive heart failure have been studied for the incidence and severity of liver damage, by a detailed clinical and liver function study and biopsy. The results are being analysed for correlation.

7. A histopathological study of the liver in chronic malaria was done in 34 cases. It was noted that malaria of a short duration could produce a tangible damage to the liver cells. The nature of changes has been discussed in detail. An important observation has been the absence of typical cirrhotic changes even in chronic recurrent malaria.

8. Fifty-one cases of leprosy for the incidence of hepatic involvement have been taken up.

9. The Registry of Hepatic Pathology has registered 309 cases till date, the most important contributions being from Dr. R. N. Chaudhuri (tropical splenomegaly) and Dr. R. M. Kasliwal (chronic amœbiasis).

On the suggestion of the Liver Diseases Sub-Committee at its meeting in June, 1954 at Coonoor, a bibliographic index of articles on liver diseases in the available journals in this country is being maintained with the co-operation of the other members of the Sub-Committee.

A critical review of the histopathological changes seen in liver biopsies in cases of infantile cirrhosis, was undertaken together with other members of the Liver Diseases Sub-Committee. This comprised of the review of literature and study of microscopic sections.

1. RÔLE OF ADRENAL CORTEX IN THE EVOLUTION OF CARBON TETRACHLORIDE INDUCED CIRRHOSIS.

It was shown by Cameron and Karunaratne (*Jour. Path. Bact.*, **42,** p. 1, 1936) that cirrhosis of the liver induced by a chronic carbon tetrachloride injury in rats was reversible up to a certain stage and failed to regress after that. In the light of the observation by Selye (*Stress*, *Acta Inc.*, Canada, 1950) that chronic stress can result in cirrhosis, and that of Hill that adrenal cortex plays some rôle in the pathogenesis of ‘ serous hepatosis ’, this study was undertaken to investigate if adrenal cortex also plays a part in the reversal of carbon tetrachloride cirrhosis.

Cirrhosis was induced in young albino rats by giving biweekly subcutaneous injections of carbon tetrachloride (0·1 c.c. per 100 g. body-weight in an equal volume of liquid paraffin). Third week onwards, a batch of seven to nine rats was sacrificed at weekly intervals to study the extent of cirrhosis. After sixth week a batch of eight rats was isolated every week, and spared of further injections for follow-up studies. These were sacrificed in two batches after two and three weeks to study the reversibility or otherwise of the cirrhotic process.

Livers were studied histologically with H and E, Masson and reticulum stains. Their quantitative collagen content was estimated chemically by the technique of Lowry *et al.* (*Jour. Biol. Chem.*, **139,** p. 795, 1941). Adrenal function in every animal was assessed by a study of the ascorbic-acid content of the adrenal by the technique of Roe and Keuther (Quoted by Hawk *et al.*, ‘ Practical Physiological Chemistry ’, 1947, p. 1137) and its capacity to deplete it when subjected to stress. This was done by exposing one or two animals with every batch of ‘forward’ experiment rats to cold by putting them on ice or giving subcutaneous injections of histamine hydrochloride (1 mg.) and ACTH (1 mg.).

These animals were also sacrificed 3½ hours later and the depletion of adrenal ascorbic acid compared to average normal, noted. In addition, one of the adrenals in every rat was also studied histologically with H and E and sudanophilic stains.

On the advice of the Liver Diseases Sub-Committee at its meeting in Baroda in November, 1954, attempt was made to include some more tests of adrenal cortical function. The 17-ketosteroid content of the adrenal was attempted by the histochemial technique of Albert and Leblond

(*Endocrinology*, **39,** p. 386, 1946) and that of urine by the chemical technique of Callow and Zimmerman ('Recent Advances of Clinical Pathology,' 1951). In the former, no good colour contrast could be seen possibly owing to the paucity of the steroid in rat adrenal. In the latter again the 17-ketosteroid content of the rat urine was found to be too low for estimation by this method as it requires a minimum of 50 c.c. of urine, whereas no more than 10 c.c. to 15 c.c. could be collected by even putting the whole batch of rats together in a bolic cage. Hence, they were given up. The study of eosinopenia under stress as a measure of adrenal function was also not feasible as the total eosinophil count of the normal rat blood is 1 to 5 cells per cm. Any diminution of the count below the normal one per cent was naturally inconceivable. Hence, only the ascorbic-acid content and its depletability under stress and sudanophilia of the adrenal cortex were stuck to for the evaluation of its function. That these are reliable indices of adrenal-cortical function in experimental study, has been observed by Sayers (' Recent Progress in Hormone Research ', Academic Press Inc., New York, 1948).

Since the rats of the 'forward' experiments were sacrificed roughly about 60 hours after the last injection, a control experiment to obtain comparable ascorbic-acid values in the normal rat after giving a similar dose of the chemical was run by sacrificing two to four animals at varying intervals of two to six hours after the injection. At 60 hours, some of the rats were again injected and others exposed to the stress of cold, ACTH and histamine. Effect of similar types of stress on normal uninjected animals was also stutied to serve as control.

Observations and conclusion.

The severity of cirrhosis was divided into five arbitrary grades ranging from + to +++++, judging from the thickness and extent of trabeculæ and degree of pseudolobulation in the reticulum stain. Table I represents the degree of fibrosis at different periods of the experiment, represented by the average number of *pluses* in a particular group correlated with the collagen content of the livers.

If was noted that except for some condensation of reticulum, there was no cirrhosis up to four weeks. At five weeks, there was a tendency to pseudolobulation, but in the follow-up studies, the bands were completely absorbed. Same qualitative changes were more or less present in the seventh and eighth week series. At ninth week, there was still appreciable regression in the second follow-up. At tenth week and later it was irreversible. By and large similar observations were made in both the histological and quantitative collagen study of the livers.

Ascorbic-acid values of the adrenals of normal young albino rats ranged from 1·23 mg. to 1·36 mg./g. with a mean of 1·3125 mg., standard deviation of 0·03441, standard error of 0·0086, and co-efficient of variation of 2·26 per cent (Table II).

TABLE I.

Showing the average grades of fibrosis and collagen content of the livers in the experimental, follow-up and cortisone groups.

(Figures in brackets indicate the range.)

Week	'FORWARD'		1ST FOLLOW-UP		2ND FOLLOW-UP		CORTISONE GROUP	
	Fibrosis (average *pluses*)	Collagen, mg./g.	Fibrosis (average *pluses*)	Collagen, mg./g.	Fibrosis (average *pluses*)	Collagen, mg./g.	Fibrosis (average *pluses*)	Collagen, mg./g.
III	0·5 (0·1)	6·44 (4·87–7·65)	...	...	...	...	...	...
IV	1·5 (1–2)	6·82 (6·42–7·23)	...	...	...	...	...	...
V	1·75 (1–2)	7·05 (5·12–9·4)	...	...	...	...	...	...
VI	2 (1–4)	8·61 (7·56–9·25)	1 (1–1)	6·61 (5·98–7·25)	0·5 (0–1)	6·44 (5·76–7·26)	...	...
VII	2 (2–2)	8·55 (7·52–9·52)	0·5 (0–1)	7·14 (6·98–7·31)	0·5 (0–1)	7·09 (6·96–7·56)	...	...
VIII	3 (1–4)	12·69 (8·9–14·89)	2·3 (2–3)	6·62 (5·12–7·31)	0·5 (0–1)	6·08 (5·21–6·95)	1·4 (1–2)	5·31 (3·38–7·32)
IX	4·1 (2–5)	17·91 (12·13-27·62)	2·5 (2–3)	8·3 (4·19–12·71)	2·2 (1–3)	7·52 (5·53–9·70)	2·3 (1–4)	7·04 (3·35–10·25)

TABLE I—(*Concld.*)

Showing the average grades of fibrosis and collagen content of the livers in the experimental, follow-up and cortisone groups.

(Figures in brackets indicate the range.)

Week	'FORWARD'		1ST FOLLOW-UP		2ND FOLLOW-UP		CORTISONE GROUP	
	Fibrosis (average *pluses*)	Collagen, mg./g.	Fibrosis (average *pluses*)	Collagen, mg./g.	Fibrosis (average *pluses*)	Collagen, mg./g.	Fibrosis (average *pluses*)	Collagen, mg./g.
X	4·3 (3–5)	21·49 (13·6-26·75)	4·5 (4–5)	20·97 (15·97-26·05)	4 (4–4)	14·51 (10·86-20·37)	3·4 (1–5)	16·08 (8·65-25·75)
XI	4·64 (4–5)	24·71 (23·3-25·72)	4·2 (3–5)	21·15 (11·38-27·41)	4 (3–5)	19·73 (10·98-30·3)	4·4 (4–5)	19·67 (15·37-28·88)
XII	5 (5–5)	26·48 (23·24-30·34)	5 (5–5)	25·6 (21·37-28·39)	4·5 (4–5)	25·5 (24·76-26·23)	4·2 (4–5)	21·46 (18·27-29·74)
XIII	4·75 (4–5)	28·61 (27·69-29·21)	5 (5–5)	27·53 (26·3–28·76)	5 (5–5)	27·92 (27·52-28·32)	4·8 (4–5)	25 (18·03-29·67)
XIV	4·85 (4–5)	31·31 (22·72-36·32)	4·5 (4–5)	30·74 (26·59-34·89)	5 (5–5)	32·83 (32·18-32·48)	4·75 (4–5)	32·75 (26·84-36·26)

TABLE II.

Showing the ascorbic-acid content of the adrenal cortex at varying periods and its depletability by various types of stress.

Number of weeks	Adrenal ascorbic acid, mg./g	Adrenal ascorbic-acid percentage of normal	DEPLETABILITY OF ASCORBIC ACID (PERCENTAGE OF NORMAL)		
			Cold	Histamine	ACTH
Normal ...	1·3125± 0·0825	100	77	52	59
First week ...	1·57±0·1	120	77·2	53·1	60
Second week	1·50±0·18	114·5	62	51	59
Third week ...	1·41±0·09	108·2	73·3	54	57
Fourth week	1·54±0·11	117·6	68	52·5	58
Fifth week ...	1·45±0·12	110·7	64	50	57
Sixth week ...	1·00±0·11	76·3	62	51	54
Seventh week	0·93±0·14	71·45	66	49	55
Eighth week ...	0·96±0·09	73·28	60	49	57
Ninth week	1·04±0·1	79·4	45	43	54
Tenth week ...	1·07±0·15	82·06	42	40	43
Eleventh week	1·18±0·15	90·67	42	22	28
Twelfth week	1·49±0·06	113·7	34·4	28	32
Thirteenth week	1·48±0·1	113·3	40	27	38
Fourteenth week	1·5 ±0·07	114	40	26	42

In an uninjected control, cold stress continued for three and a half hours resulted in an average of 77 per cent depletion, whereas with carbon tetrachloride it was only 35·2 per cent, thus showing that the latter is a weak stressor. Histamine and ACTH produced 52 per cent and 59 per cent depletions, respectively.

In rats receiving a single injection of carbon tetrachloride after the initial fall at three and a half hours, the ascorbic-acid values started rising and at 60 hours, they averaged 1·8 mg./g., e.g., 141·2 per cent of average normal. Cold stress again given at this stage resulted in the lowering of ascorbic-acid levels to 0·49 mg., e.g., 73·95 per cent depletion, whereas a single injection of carbon tetrachloride resulted in 24 per cent depletion, again showing that this chemical is a poor stressor.

Table II gives the ascorbic-acid levels at different stages of the cirrhotic process and changes in their depletability by the three types of stress.

Generalizing from the above data the observations can be divided into four phases:—

Phase I: No cirrhosis.—This extends roughly up to the fifth week. The ascorbic-acid levels were significantly higher as compared to the average normal, i.e., more than 3 S.D. higher than the normal mean. They were also capable of being induced to normal depletability on being exposed to all the three types of stress.

Phase II: Reversible cirrhosis.—This extends from sixth to eighth week. In this phase, the ascorbic acid values were significantly lower than the average normal. The stresses could still deplete the ascorbic acid to the normal extent.

Phase III: Irreversible cirrhosis.—This extends from ninth to eleventh week. The average ascorbic-acid values were still lower than normal, but the various stresses caused significantly lower depletions. This was most marked by histamine, although a similar trend was noticeable with ACTH.

Phase IV: Irreversible and (?) *progressive cirrhosis.*—This extends from the 12th to 14th week. In this phase, the average adrenal ascorbic-acid values were normal but the depletability continued to be low.

The histological picture of the adrenal was also well correlated with the ascorbic-acid values. In the early stages, e.g., up to fifth week, the adrenal cortex was hypertrophied involving chiefly the zona fasiculata which was almost three times as thick as the zona reticularis compared to the even thickness of both the zona in a normal adrenal. This was associated with increased sudanophilia and clear cytoplasm in the big cells, and high ascorbic-acid values.

The above observations seem to indicate that the adrenal cortex is related to the reversibility of cirrhosis induced by carbon tetrachloride.

A non-cirrhotic liver or a liver with cirrhosis which is still in the reversible phase, is associated with a normally functioning adrenal which is capable of recovering from stress in 60 hours and depleting further its ascorbic acid content when subjected to further stress. When the cirrhosis enters the irreversible phase, the adrenal cortex is noted to become sluggish and incapable of further augmenting its function on exposure to new stress.

It is felt that carbon tetrachloride results in the production of certain factors locally which induce proliferation of connective tissue elements. This proliferation seems to be counteracted in the early stages at least by factors, one of them being the adrenal corticoids, which are known for this effect. In the early stages, the adrenal is capable of coping with this stress, but when it is continued recurrently, the adrenals find it more difficult to recover. Later carbon tetrachloride excites the general adaptation syndrome less effectively, owing to the fact that either it acts less effectively on the tissues which acquire adaptation, or that some block has occurred in the pituitary adrenal axis or that the adrenals have become non-responsive. The observation, that depletability by both histamine and ACTH is affected, seems to indicate that there is some change both at the pituitary as well as adrenal cortex level. Owing to this, the connective tissue proliferation progress remains unabated and the fibrosis becomes irreversible.

II. ACTION OF CORTISONE ON THE PROGRESS OF FIBROSIS OF THE LIVER INDUCED BY CARBON TETRACHLORIDE.

In view of the above observations of the association of a normally functioning adrenal with the reversible stage of cirrhosis and two conflicting reports in literature (Aterman, *Arch. Path.*, **57,** p. 12, 1954 and Diengot, *et al.*, *Arch. Path.*, **58,** p. 449, 1954) regarding the action of cortisone on the progress of fibrosis, another corollary in the above experiment was planned. After eighth week, when cirrhosis had well set in, a group of six rats were set aside every week and given daily intramuscular injections of cortisone acetate averaging 7 mg. a day per rat for ten days. These rats were sacrificed after another four days, to time with the two week follow-ups which served as the controls, and the degrees of fibrosis in the livers were compared.

Table I gives the figures of average severity of fibrosis and collagen content of the livers of the cortisone group. Comparing with the first follow-up, it is seen that cortisone did have some ameliorating effect on the cirrhotic process in the eighth and ninth weeks. Particularly, in the eighth week, in the cortisone batch, the fibrosis was of a distinctly lesser severity and the livers were closer to normal than the first follow-up. The difference became less and less obvious from tenth week onwards. In the later stages, there was practically no difference in the cortisone and follow-up groups.

Thus it was clear that cortisone at least did not have an enhancing effect on cirrhosis. It accelerated the reversal when the process was still in the reversible stage. It is felt that cortisone only helps reversing certain stages in the maturation of collagen like the type of change from precollagen to collagen or polymerization of collagen. Once mature collagen is laid down, cortisone cannot undo it. Cortisone seems only to accelerate the natural turnover. It cannot possibly induce the turnover, where there is none.

III. A STUDY OF THE RELATION OF THE ADRENAL CORTEX TO ACUTE CARBON TETRACHLORIDE HEPATOPATHY.

Carbon tetrachloride has been reported to induce a rise in the free amino acids of the liver in the prenecrotic phases (our last year's report) and a marked reduction in its glutathione content six hours after the administration (Patwardhan *et al.*, *Ind. Jour. Med. Sc.*, **7,** p. 633, 1953). In states of alarm reaction of a non-specific nature, the amino-nitrogen of the liver has been known to increase (Russel and Long, *Amer. Jour. Physiol.*, **147,** p. 175, 1946). Similarly, Larget *et al.* (Quoted by Selye, *Stress Acta Inc.*, Canada, 1950) have shown that glutathione is reduced in states of surgical shock, and Conn *et al.* (*Jour. Lab. Clin. Med.*, **34,** p. 255, 1949) have demonstrated a reduction of glutathione with ACTH. Correlating these facts, we wondered if, in our observations of the amino-acid changes in the liver after a single injection of carbon tetrachloride which is known to be an activator of the G.A.S., the activity of the adrenal cortex was partly or wholly responsible. Hence these experiments were run to investigate whether the changes observed above were incidental to the adaptation or due to a specific action of the chemical.

Several rats were given a single injection of carbon tetrachloride (1·8 c.c./kg. body-weight) and sacrificed in batches of four to six animals each at intervals of 2, 4, 6, 12, 18 and 24 hours later. Apart from a routine histological study of the liver, the total protein nitrogen and free amino acids (' Kjeldahl Technique ', King, ' Microanalysis in Medical Biochemistry ') and glutathione content (Grunert and Phillip, *Arch. Bioch.*, **30,** p. 217, 1951) of the liver were estimated. Simultaneously, the adrenal activity was assessed by chemical estimation of the ascorbic-acid content (Roe and Keuther, *loc. cit.*) and histochemical study for sudanophilia (Chifelle and Putt, *Stain Technol.*, **26,** p. 51, 1951). Normal rats were used for establishment of a normal pattern.

TABLE III.

Showing the levels of total proteins, amino acids and glutathione content of the liver and ascorbic-acid content of the adrenal at varying intervals after a single injection of carbon tetrachloride.

(Figures in brackets indicate the range.)

Time in hours after the injection	Total proteins (liver), g. per cent	Free amino acids (liver), g. per cent	Glutathione (liver), mg. per cent	Ascorbic-acid (adrenal), mg. per cent
Normal uninjected rat	21·8 (18·8—24·1)	0·99 (1·23—1·36)	216·2 (115—300)	1·3125 (1·23—1·36)
2	23·56 (23·1—24·1)	0·935 (0·97—0·9)	211·5 (189—236)	1·33 (1·32—1·34)
4	21·575 (20·9—22·4)	0·965 (0·89—1·14)	172·3 (93·3—222·9)	0·96 (0·89—1·1)
6	21·9 (21·3—22·5)	1·25 (1·1—1·4)	99 (91—108)	1·355 (1·3—1·48)
12	21·5 (21·0—22·0)	1·22 (0·99—1·47)	224·02 (209—240)	1·535 (1·24—1·8)
18	20·85 (18·7—22·3)	1·61 (0·98—2·5)	154 (100—240)	1·58 (1·3—1·8)
24	22·5 (21·7—23·1)	1·962 (1·45—2·8)	118·75 (133—150)	1·645 (1·5—1·78)

As is apparent from Table III, the total protein nitrogen did not show any appreciable change throughout the study. The free amino acids showed a tendency to rise at four hours and reached the peak, 18 to 24 hours after the injection. Glutathione showed a tendency to fall at four hours, and was lowest at six hours. The ascorbic-acid levels of the adrenal were lowest at four hours and tended to rise at subsequent periods, reaching a peak at 24 hours.

Histologically, in the liver, there was a minimal centri-zonal necrosis at 6 to 12 hours, followed by the formation of mid-zonal 'balloon-cells' at 18 hours and massive necrosis. The adrenal showed diminution of sudanophilia at the four-hour stage followed by slow accumulation of the sudanophilic material and hypertrophy of the adrenal cortex.

Judging along the observations of Sayers ('Recent Progress in Hormone Research', 1948) low concentration of ascorbic acid in the adrenals, along with diminished sudanophilia is a sign of adrenal activity. In the present study this was reached at four hours. The peak of changes in the free amino acid and glutathione concentration in the liver cells was arrived at 24 hours and 6 hours after the injection, respectively. Whereas the adrenal function seemed to have returned to normal from the six-hour stage onward, the changes in the amino-acid and glutathione levels persisted or even progressed. These facts seem to indicate that the state of adrenal activity dose not bear a simple cause and effect relationship with the changes in the liver cells. This study, therefore, seems to add to the evidence presented in our last year's report, that the action of carbon tetrachloride on the hepatic cells is a direct one and the free amino-acid changes are not related to the adrenal activity.

IV. MODE OF ACUTE INJURY TO THE HEPATIC CELLS BY CARBON TETRACHLORIDE.

During the course of study of acute carbon tetrachloride hepatic injury in the liver, reported in detail last year, it was noticed that the alkaline phosphatase diminished in the central zones of the hepatic lobule in the prenecrotic stages. To investigate, whether this action on the enzyme was chemical or metabolic, slices of the liver of four normal rats were fixed in acetone each containing varying concentrations of carbon tetrachloride (2 c.c./100 c.c., 5 c.c./100 c.c., 10 c.c./100 c.c. and 15 c.c./100 c.c.). Control slices from same animals were fixed in pure acetone. These were treated and developed identically for alkaline phosphatase by Gomori's technique (*Jour. Lab. Clin. Med.*, **37,** p. 526, 1951).

No difference in the intensity or distribution of the stain was noted in two sets.

In a second experiment, serum alkaline phosphatase was estimated in nine specimens of normal blood (Bodensky, *Jour. Biol. Chem.*, **101,** p. 93, 1953). To the same serum varying concentrations (one to five drops to 1 c.c. of serum) of carbon tetrachloride were added and the mixture incubated at 37° C. for two hours. This again did not seem to interfere with the activity of the enzyme, as identical values were obtained in the two sera.

The above evidence adds to the belief that the action of carbon tetrachloride on the hepatic cell is purely metabolic.

V. TO STUDY THE RÔLE OF NECROTIC TISSUE IN THE PRODUCTION OF ENZYME CHANGES IN THE LIVER IN ACUTE CARBON TETRACHLORIDE INJURY.

In a histochemical study of the liver after a single injection of carbon tetrachloride reported in detail last year, it was noted that 12 hours onwards, the alkaline phosphatase reaction was intensified in the peripheral 1/3rd of the lobule. Cameron and Oakley (*Jour. Path. Bact.*, **38,** p. 17, 1934) have studied the effect on unboiled and boiled liver extracts on the connective tissue proliferation and have found differences in their response. It is possible that necrosed tissue in which the various enzymes have been affected is comparable in some ways to boiled liver. Menkin (quoted by Stowell and Lee, *Arch. Path.*, **50,** p. 519, 1950) has shown some change in the liver cell injected with 'necrosin'.

The present study was undertaken to investigate the rôle of necrotic tissue present in the central one-third of the lobule in initiating these changes.

Necrosis was first induced in the liver by the administration of carbon tetrachloride to the normal albino rats. The dose consisted of about 1 c.c. subcutaneously per rat, followed by another 1 c.c. after 24 hours. A few experiments with higher doses were also empirically performed. The rats were sacrificed after another 24 hours, and a frozen section of the liver studied to ensure that a good deal of the liver had been necrosed. In none of them could the whole liver be necrosed. There was always a peripheral rim of surviving cells.

Two pieces of necrosed liver, each weighing about 2 g. were taken from each specimen. One piece was thoroughly homogenized and 25 c.c. of aqueous extract prepared from it. To the other piece, similarly homogenized and extracted, solutions of sodium chloride (five per cent) and ammonium sulphate (50 per cent) were added in order to precipitate the proteins, and a non-protein containing aqueous extract was thus obtained. Various volumes, e.g., 0·5 c.c., 1 c.c., 1·5 c.c. and 2 c.c. of each type of extract were injected subcutaneously into batches of two rats each. They were sacrificed in two lots at 12 hours and 24 hours. These livers were studied for the alkaline-phosphatase activity.

No change on the alkaline-phosphatase activity was observed in any of the livers. It appears, therefore, that necrosed tissue does not directly contribute material responsible for the increased peripheral reaction of alkaline phosphatase in acute carbon tetrachloride injury.

VI. HISTOPATHOLOGY OF THE LIVER IN MALARIA.

Thirty-four cases of malaria with a history ranging from two weeks to twenty years were studied by a detailed liver-function study and simultaneous liver biopsy.

Reticulo-endotheliosis was the commonest pathologial change observed, being present in varying degree of severity in all cases. Diffuse parenchymal damage to the liver cells was present in some degree in 30 cases. Twenty-three cases showed varying degree of cytoplasmic rarefaction in the liver cells, the change being fairly severe in a few cases. Only seven cases showed some periportal cellular infiltration or portal fibrosis. Typical cirrhotic changes were not encountered.

It was concluded that malarial fever even of a short duration can bring about a parenchymal damage to the liver of moderate severity, which becomes evident as a temporary functional derangement. The exact mechanism by which this damage is produced is not known. It could be produced by some kind of toxin, hyperthermia of malarial episodes, anæmia or malnutrition or a combination of the above factors.

Malaria by itself even though recurrent and of long standing does not produce a typical cirrhosis and hence use of the term malarial cirrhosis is deprecated.

VII. Liver function studies in leprosy.

Till date 51 cases of leprosy have been taken up and a detailed liver function work done. Of these, only 12 are of the lepromatous variety. The only signs of aberrated liver function have been met with in some lepromatous cases. The study is being continued.

VIII. Liver function studies in congestive failure.

Thirty-six cases of congestive heart failure due to various causes have been collected till date. The results are being analysed for correlation between the severity of heart failure and the degree of functional damage and pathological changes in the liver.

IX. Registry of hepatic pathology.

Till date 309 cases have been registered, including a good few from abroad. Every slide has a detailed histological description and the Registry's diagnosis, a copy of which is sent to contributors. A disease-wise index of all slides has been prepared.

A comprehensive index of articles on liver diseases in children from 1916 to 1952 was prepared last year. The Registry is also maintaining, with the help of Dr. V. R. Khanolkar, Dr. V. N. Patwardhan, Dr. B. K. Aikat, Dr. P. Kutumbiah and Dr. M. V. Radhakrishna Rao, a bibliographic index of articles on liver diseases, available in India. Cyclostyled copies of the till-date collections have been circulated to the members of the Cirrhosis Sub-committee. In addition, the articles are being card-indexed for future reference and use of any worker on liver disease.

The Cirrhosis Sub-committee at its meeting in June, 1954 at Coonoor, prepared a set of slides of liver biopsies from cases of infantile cirrhosis met with in different parts of the country, specially contributed by Drs. B. K. Aikat, Achar and Kutumbiah. Together with other workers, these slides were critically reviewed in the liver of the various clinical stages of the diseases. The detailed report has been submitted to the Secretary, Indian Council of Medical Research and is being incorporated in a memoir prepared under its auspices.

The two most important sets of contribution have been made by Dr. R. M. Kasliwal (29 cases) and Dr. R. N. Chaudhuri (22 cases) on chronic amœbiasis and tropical splenomegaly, respectively. They are all specimens of liver biopsy with short clinical histories. These slides have been studied and the opinion of the registry submitted to the contributors.

7. Inquiry on electro-retinography in Vitamin 'A' deficiency under Dr. R. P. Dhanda at the Mahatma Gandhi Memorial Medical College, Indore.

Simultaneous Recording of Electro-retinograms and estimation of Vitamin A in Plasma was carried on during the year in cases of clinical menifestations of Vitamin A deficiency.

Fasting samples of Blood of 13 Normal Indian adults over the age of 15 years were analyzed for Vitamin A content by the Antimony Tricholoride method on a Photo-electric colorimeter. The results varied from 100 to 192 I.U. of Vitamin-A/100 c.c. of Plasma, an average of 150 I.U. Similarly in the blood of 12 Normal children below the age of 15 years the Vitamin-A content averaged 36 I.U./100 c.c. of Plasma (the explanation of a low figure in children is suggested during the Text of the report below).

A summary of the results of Vitamin-A estimation and the Electro-retinographic studies is given in the following table which is self explanatory—

Clinical condition	Electro-retinography		Vitamin-A in blood	
	Number of patients investigated.	Average b-potential in mV.	Number of cases	Average Vitamin-A in I.U./100 c.c. of Plasma
Normal adults Co-op. +++ or more	19 eyes of 11 pts.	·32mV.	13	150
Normal children above 7 Co-op. +++ or more	11 eyes of 8 pts.	·31mV.	9	39
Normal children below 7 (General Anæsthesia)	6 eyes of 6 pts.	·26mV.	3	26
Xerosis Adults Co-op. +++ or more	16 eyes of 11 pts.	·29mV.	8	103
Xerosis children above 7	9 eyes of 6 pts.	·24mV.	5	32
Xerosis children below 7	6 eyes of 6 pts.	·18mV.	3	20
Xerosis with Night Blindness in Adults	19 eyes of 11 pts.	All but 5 Extinguished	3	33
Xerosis with Night Blindness in children above 7	11 eyes of 9 pts.	All but 3 Extinguished	8	15
Xerosis with Night Blindness in children below 7	30 eyes of 23 pts.	All but 3 Extinguished	6	15

Excusing myself for the repetitions of some of the observations during the last two years' reports, the following observations are a summary description of the results of this enquiry.

OBSERVATIONS.

Xerosis.—It is apparent from the above observations that Xerosis behaves differently in adults and children. For example Xerosis in one particular case has existed for 15 years without ever causing any defect in Night vision and the patient came to the hospital only for cosmetic reason. Again in a case, Xerotic patches in both eyes has been known to exist for five years, yet she had a b-potential of ·257 and ·236mV. in each eye. Yet again another case aged 41 years had very big Xerostic patch which I have graded as +++ in each eye. The patient had observed them for over two years and yet the b-potential was ·237 and ·330 mV. A fourth case, with Double Bilatial Xerotic patches for three to four years, had a b-potential of ·39 and ·4 mV.

Similarly there have been cases of Xerosis graded as ++ or more with Vitamin-A contents of 125, 130, 185 I.U./100 c.c. of Plasma. It has also been observed tht Xerosis has existed in the conjunctiva of adults of even upper economic status where not only the food part is satisfactory but the General condition is also above an average. Response to treatment is also very poor e.g. in a case, Xerosis had been known to continue to exist for years together even though patient had 60 injections of "massive-A" over a period of nine months. Electro-retinogram remained unaltered.

In case of children, the observations were some what different. Xerosis has usually existed for less than six months before they attended the hospital. Again most of the children who come with Xerosis, clinical malnutrition is quite apparant and children usually, belong to a poor class of society. In good percentage of cases Xerosis has followed a gastro intestinal upset. b-potential not only tends to be sub-normal but was found completely extinguished in 5 out of the 27 cases even though there was no Night Blindness. May be that these cases were border line cases which if left untreated would have very soon developed Night Blindness.

Response to treatment is also quick in children so that big Xerotic patch has been made to disappear with an average of six injections of Massive 'A' I.M. every 4th day.

Night Blindness.—Children below the age of 15 years are particularly prone to develop Night Blindness as a result of Vitamin-A deficiency. This is born out by the large number of out patients brought to the Ophthalmic Hospital ill nourished and responding to treatment with Vitamin-A.

Again the Electro-retinogram was mostly either extinguished or b-potential was extremely small. Treatment of these cases resulted in rapid disappearance of Night Blindness but Electro-retinogram was comparatively much slower to return while Xerotic patches took still longer to disappear.

As expected, the treatment in case of children was much more rapidly effective both with regard to clinical symptoms as well as the return of Electro-retinogram.

Contrary to the above, Night Blindness is not a common feature of Vitamin-A deficiency in case of adults, only 13 cases have been come across over a period of nearly two years out of thousands of out patients at the Ophthalmic Hospital, Indore (India). In these Night Blindness was of an acquired nature and other causes of Night Blindness has been definitely excluded.

DISCUSSION.

Some observations deserve a more detailed discusson.

A normal Electro-retinogram in all cases of Xerosis in adults so long as it is Xerosis alone is a very interesting finding. The interest is further heightened by a nearly normal if not completely normal Vitamin-A content of their blood. This raises a pertient question whether a Primary Xerosis in the absence of any other cause in conjunctival sac is a direct result of Vitamin-A deficiency or some indirect menifestation. This mystery is again heightened by poor response of Xerotic patches in adults to Vitamin-A administration. Whatever relation exists between Vitamin-A content and Xerosis, is only indicated by the existence of Xerosis in children who if untreated very soon develop Night Blindness. The only conclusion that can be drawn from the observations and investigations of a very large number of cases with Xerosis leads one to conclude that Xerosis is not a direct menifestation of Vitamin-A deficiency but is the result of some other indirect or allied cause which has a close association to the Vitamin-A content in Blood. What that factor is, can not be deducted from these investigation but it certainly opens up a field for further research of very interesting nature.

Extinguished Electro-retinogram in cases of Night Blindness due to Vitamin-A deficiency has been demonstrated for the first time during these investigations. Prof. Karpe in his personal communication to the author has only casually referred to the observation of an extinguished Electro-retinogram in a single experimental case of malnutrition. These investigations have conclusively proved that onset of clinical menifestation of Night Blindness due to Vitamin-A deficiency is a deciding factor about it effects on Electro-retinogram. The Electro-retinogram gets suddenly extinguished as soon as the Vitamin-A content of Blood falls to a level that it causes clinical menifestation of Night Blindness.

The above observations indicate that so long as a person has only Xerosis, not only the Vitamin-A content of the Blood is at a respectable level but his Electro-retinogram also remains unaffected, but as soon as he developes Night Blindness, the Electro-retinogram gets completely extinguished and Vitamin-A level in Blood falls to a minimum thresh-hold.

Another observation that deserves discussion is increased sensitivity of children to Vitamin-A deficiency. It is obvious from the above observation that Vitamin-A content of Normal children below the age of 15 years is definitely of much lower order than that of adults. This may be due to a lower intake and more probably due to a bigger demand during the developing stages of life. That also probably explains the greater frequency of Night Blindness in children due to Vitamin-A deficiency. While in adults the demand being much less the Vitamin content of Blood does not fall to a level low enough to cause Night Blindness.

Again finding of extinguished Electro-retinogram in five cases of Xerosis alone in children below the age of seven years suggests that these children had reached a critical stage of Vitamin-A content and would very soon have developed Night Blindness if left untreated. The Vitamin-A content of one of these cases was only 17 I.U. This and the General finding of lower Vitamin-A content in children explains the higher sensitiveness of children to Vitamin-A deficiency and also why most of them very soon develop Night Blindness.

The minimum Vitamin-A thresh-hold in blood must obviously be extremely low because cases have been observed where Vitamin-A content had fallen to as much as 50 I.U./100 c.c. of Plasma, yet the Electro-retinogram remained normal and the patient never developed Night Blindness. In cases of Night Blindness not only the Vitamin-A content is invariably low but the Electro-retinogram was also extinguished. The observations regarding Vitamin-A estimation are made with reservation and full realization of the fact that chemical analysis of Plasma for Vitamin-A gives very variable results. The value attached to the chemical analysis during these investigations is a relative one but is considered of value because analysis was done under similar circumstances in all cases in that Blood collected was on a fasting stomach, Blood was analysed within three hours of its collection, and the chemical method adopted as well as Photo Electro-colorimeter used was the same during the whole period of Research work.

Incidently these investigations throw an interesting light on the pathogenesis of Night Blindness and its association to an extinguished Electro-retinogram. The degeneration of the Retina as a whole in Retinitis Pigmentosa resulting in an extinguished Electro-retinogram is apparently not difficult to understand. A hypothesis put forward by Lorren A Riggs in his paper on " Electro-retinogram in cases of Night Blindness due to Retinitis of Pigmentosa or of congenital origin " has suggested the existence of " holes in the Retina " as the mode of an extinction of Electro-retinogram in these cases. Observations set forth in this paper obviously prove that this hypothesis need not necessarily be correct. As is shown by these investigations, an Electro-retinogram can get completely extinguished even though Retina may structurally be normal, and only functionally affected as in case Vitamin-A deficiency.

Structural change in the Retina for an Electro-retinogram to be extinguish is also disproved by the observation that a Retina which has lost its electrical response completely may regain it fully with treatment with Vitamin-A. This leads to the conclusion that no organic change need necessarily be present for Electro-retinogram to be extinguished and proves that an Electro-retinogram represents the functioning capacity of Retina rather than the structural morphology and again that it is a reversible phenomenon.

CONCLUSIONS.

1. An Electro-retinogram can be successfully recorded in most of the human subjects above the age of seven years while general anesthesia is usually necessary below that age.

2. b-potential of a Normal Indian adults is ·32mV. and that of a child between ages of 7-15 years is ·31mV. while below the ages of seven years ·26mV.

3. Electro-retinogram remains normal so long as patient suffers from Xerosis alone and the Vitamin-A content of Blood is above the minimum thresh-hold level.

4. Electro-retinogram gets completely extinguished as soon as the Vitamin-A deficiency leads to the menifestations of Night Blindness and the Vitamin-A content falls below the minimum thresh-hold level.

5. Absolute minimum Vitamin-A requirement in human beings must be of a very low order and Vitamin-A thresh-hold is in the neighbourhood of 20 I.U./100 c.c. Plasma in children below 15 years age and a little higher in adults.

6. Children are much more susceptible to Vitamin-A deficiency as compared to Adults and develop Night Blindness as well as lose the electrical response in the Retina much more rapidly than in adults.

7. Night Blindness as a menifestation of Vitamin-A deficiency is much more frequent in children than in adults.

8. Children respond to treatment much more rapidly than adults.

9. Xerosis is an indirect menifestation of Vitamin-A deficiency rather than a direct one.

10. While Night Blindness is a direct result of Vitamin-A deficiency.

11. Functional defect in Retina is enough to lead to an extinguished electrical response of Retina and a patholgoical and structural change is not a necessity.

12. An extinguishd Electro-retinogram is a reversible phenomenon.

However, due to the very late arrival of Adaptometer from Germany a small part of this enquiry has yet to be completed and that is to support the above observations with the help of simultaneous records of dark adaptation.

STUDY OF DARK ADAPTATION.

From the small number of cases already investigated, one thing can be stated that nearly normal dark adaptation in cases of Xerosis simply supports the conclusions already submitted that Xerosis is not a direct result of Vitamin-A deficiency, but indirect one. However, further investigations are necessary to confirm this conclusion regarding dark adapatation.

8. Inquiry into endemic focus of schistosomiasis in India under Dr. R. K. Gadgil and Dr. S. N. Shah at the Grant Medical College, Bombay.

During the year a large portion of work was devoted to maintaining, breeding and infecting the snails of species: (1) *Paludomus obesa*, (2) *Ferrissia tenuis*, (3) *Indo-planorbis exhustus*, (4) *Planorbis torquis*, (5) *Planorbis helisoma*, and (6) *Limnæa luteola*. By the end of the year we had been able to infect *Ferrissia tenuis* and obtain a few brevifurcate *furco-cercariæ* with a morphology similar to cercariæ of *Schistosoma hematobium*. The attempt to infect laboratory animals with these cercariæ had failed. The methods of maintaining, breeding, and infecting the snails were perfected and hence it was possible to obtain regularly a considerable number of cercariæ and proceed with intensive animal infection experiments. In current year, we have been able to infect white mouse successfully and obtain an adult pair of schistosomes with a morphology similar to *Schistosoma hematobium.* The experiments of infecting the snails of species other than *F. tenuis* have so far always been unsuccessful. Thus, we have been able to demonstrate the complete life-cycle of this schistosome only through *Ferrissia tenuis.* At present we are trying to obtain a large number of adult worms in experimental animals for study of their morphology and systematic position.

Maintenance and breeding of snails.—The following method was found to be the best for breeding and maintenance of snails: Tap water with addition of common salt 2 g. per litre of water is used in the aquaria. Stored dry decaying mango-leaves and vegetation, such as allisnaria, hydrila, etc., are added, in a small quantity, to aquaria. Boiled lettuce leaves are added every alternate day. Continuous æration with compressed air, supplying about one litre per minute for small aquaria and five litres per minute for large aquaria is maintained. The aquarium becomes ready in about week's time when algæ starts growing on the inner walls of the aquarium. Aquaria are kept in open air in diffused sunlight and the temperature varies from 79° F. to 90·8° F. in summer and from 72·2° F. to 87·4° F. in winter. The special artificial food-bemax one per cent, dried milk 0·5 per cent, powdered dried lettuce one per cent and sodium alginate one per cent—as advised by O. D. Standen in his paper, fails to attract any of the above-mentioned snails in our laboratory.

The breeding of *F. tenuis* was good. It seems that *Paludomus obesa* refuse to breed in captivity under laboratory conditions. The breeding of *Indo-planorbis exhutus*, *Planorbis torquis* and *Planorbis helisoma* is prolific. The breeding of *Limnœa luteola* has not been taken into hand as yet. *F. tenuis* is oviparous and only one egg is laid in an egg-mass. Eggs are laid on dried decaying mango-leaves and are embedded in gelatinous masses.

Infection of snails.—Two hundred to four hundred snails are taken in a glass-aquarium, 5″ × 5″ × 8″, containing about 1,500 c.c. of water, with decaying mango-leaves. A dose of infection of three to four miracidia per snail is given under continuous æration.

The following methods were tried with negative results as most of the snails died: Individual infection was given in a Kahn tube, with a dose of three to four miracidia. Mass infection with a dose of three to four miracidia per snail was given in glass-aquarium 4″ × 2″ × 2″. Mass infection with a dose of a large number of miracidia was given.

Results with various snails.—Only *F. tenuis* was proved to be a vector as the snails emitted cercariæ at the end of 4 weeks. The remaining above mentioned snails were proved insusceptible to infection. The rate of mortality of infected snails was high, especially in case of *Ferrissia tenuis.*

As *F. tenuis* is proved to be a vector of infection, the anatomical study of the same was suggested and is under progress. Not much data are available in this regard so far.

Emission experiments.—The snails which survive the period of four weeks and more, after the infection, are screened for cercarial emission at the end of four weeks. The infected snails from different aquaria were transferred to test-tubes containing aquarium water. The temperature of the water in these test-tubes was kept constant at 27° C. under fluorescent light for three hours. The snails emit cercariæ intermittently and not every day.

Animal infection.—The animals used in the infection experiments are white mice and golden hamsters. The white mouse, after shaving hair from its abdomen, was kept in a small glass aquarium 4″ × 2″ × 2″ and a cercarial suspension was added to the aquarium. The mouse was prevented from coming out of the aquarium and was exposed to infection for one hour. The mouse thus infected showed a pair of adult schistosomes in the liver after three months.

Other methods.—The mouse is tied on a wooden board with holes, with its back in contact with the board. The tail was dipped in a test-tube containing cercarial suspension for an hour. In another experiment a drop of cercarial suspension was kept on its shaved abdomen. As the tying of the mouse was uncomfortable to it, it was advised to be wrapped in a gauze 3″ wide and was placed in the desired position with an adhesive tape.

At present hamsters are being infected by keeping in a glass-aquarium 4″ × 2″ × 5″, containing cercarial suspension for one hour. Prior to exposure to infection hamster is kept in the aquarium with tap water. This was done to remove fæces and urine which it passes as a result of placing it in water.

Preparation of antigen from snails of this disease is under way and the antigen is likely to be ready before the end of the year, if infective material from animals is available in sometime from local veterinary college.

The effect of light, temperature and salinity on the hatching of ova of *S. hematobium* was studied. The data collected are under study and compilation. Different techniques of preparing permanent mounts of ova, miracidia, cercariæ and adult worms were tried. The study of the pair of adult worms, obtained from an infected mouse in the laboratory, was done. The worms resemble those of *Schistosoma hematobium* but further morphological study is still in progress. Ecological study of the snail *F. tenuis* and the effect of copper sulphate poisoning on *F. tenuis* is being studied. The study of miracidia and cercariæ, with the phase-contrast microscope, is also being done.

The number of aquaria was increased. Infection of a large number of snails *F. tenuis* was done to get carcariæ for infection of animals.

A clinical survey of the endemic focus at Gimvi (Ratnagiri District) was done in the month of February, 1955. Survey of the village Malsana (Nasik District) was done in March, 1955, from where an autochthonus case of schistosomiasis came under our observation. A detailed survey of Malsana was carried out in the month of August 1955 with negative results. The possibility that there might be an endemic focus of schistosomiasis at Malsana is, however, not given up so far, till alternate source of infection can be established. The survey of Gimvi and Malsana and eradication of the endemic focus at Gimvi was done by the Public Health Department, Poona, in the month of April, 1955.

9. Studies of the Indian poisonous snakes under Dr. P. J. Deoras at the Haffkine Institute, Bombay.

This inquiry had been sanctioned to study the habits of the common poisonous snakes of India with a view to keep them in natural surrounding of a snake farm, so that they may breed or by living longer continue to give more venom than the snakes kept in cages in the laboratory. During the pendency of this inquiry a snake farm has been established where cobra, krait, and Russell's viper snakes have been housed. The echis snake had not been housed because its habits were not known. Secondly, Russell's viper, krait and cobra snakes in the farm have bred, though not prolifically, but it was found difficult to rear the young ones. The sexual cycle during different months of the year remained to be studied. Thirdly, the venom output of the snakes kept in the farm was seen to be better, but this needed statistical confirmation by taking individual venom output.

During 1954-55, the studies in the above inquiry were directed towards the keeping of cobra, krait and Russell's viper snakes in the farm, the food habits of echis snakes, breeding habits of snakes in general and some biochemical studies. In the present studies during 1955-56 attention has been given to (*i*) the field observations on the habits of echis snakes, (*ii*) the collection and analysis of the data in connection with the venom output of cobra, krait and Russell's viper snakes in the farm as compared to the same kept in the room, and (*iii*) the study of the breeding habits of snakes in general.

1. FIELD OBSERVATION.

Field observation studies were conducted at Deogad, a Taluka in Ratnagiri District at three different times of the year on echis snakes found in the locality. These studies were done for one month at a stretch in November 1954, February 1955 and August 1955, i.e., winter, beginning of summer and rainy months. The Senior Field Assistant of Indian Council of Medical Research was posted on the spot and he had to move out at least four miles a day making observations and collections. He walked a specific distance each day on specified timings and turned stones or shook bushes on the way. Any snake found there was collected along with other animal life under the stone and bush. Names of the different plants in the locality during the period were recorded. The snake, if recovered, was taken to the headquarters and the contents of the digestive tract recovered after proper dissection. The dead animals in the gut contents were then identified and correlated with the live ones found near the places where echis was observed. The most predominant animal found in the digestive tract of the dissected echis was later collected alive and echis snake reared in the provisional laboratory in the field on the same.

Table I gives the list of animals recovered near the habitat of echis snakes and the frequency of their collections during October, February, and August months. Table II gives the details of the animals or their parts recovered from the gut contents of the dissected echis snakes. From these Tables it is seen that the majority of animals recovered and the majority of dead bodies in the gut are insects. It was possible to keep a large number of echis snakes alive in the provisional laboratory at Deogad on these insect foods. The preponderance of the different orders of insects is given below in order of merit:—

Dermaptera
Orthoptera
Coleoptera
Hymenoptera
Hemiptera
Lepidoptera

TABLE I.

Collection of echis and its food.

Date of observation	Number of miles covered for turning stones	Time of observation, hour	Number of stones turned	Number of echis recovered	Average echis caught per day	Different animals under stones turned	Different animals where echis recovered
25– 9–54 to 22–10–54 (28 days)	60	15 to 18	5,700	9	0·3214	Dermaptera Orthoptera Coleoptera Isoptera Hemiptera Lepidoptera Hymenoptera Archnida Scolopendra Millipedes Oligochæta Frogs Lizards Snakes	Small and big black beetles.
13–2–55 to 22–2–55 (10 days)	25	8 to 12 and 14 to 18	10,900	4	0·4	Orthoptera Coleoptera Hemiptera Hymenoptera Arachnida Centipedes Small mouse	Cockroach, and black beetles

TABLE I—(*Concld.*)

Date of observation	Number of miles covered for turning stones	Time of observation	Number of stones turned	Number of echis recovered	Average echis caught per day	Different animals under stones turned	Different animals where echis recovered
13–8–55 to 12–9–55 (31 days)	89·25	7 to 12 only	23,750	61	2	Annelida Dermaptera Orthoptera Arachnida Coleoptera Amphibia Reptilia	Orthoptera Small frogs Black beetles

TABLE II.

Gut contents of echis collected on the spot.

Date of observation	Number of echis recovered	Number of echis dissected	Œsophagus contents	Stomach contents	Intestine contents	Rectum contents
25–9–54 to 22–10–54	9	9	Few small nematodes	Cestodes	Nematodes Cestodes	Bits of Dermaptera. Hymenoptera. Isoptera. Orthoptera. Coleoptera. Hemiptera. Millipedes. Centipedes. Dark earthy matter.
13–2–55 to 22–2–55	4	4	*Nil*	Few insect parts seen, as terga.	By the side of the intestine 2 developed eggs. seen.	Earthy matter and a few insect parts.
13–8–55 to 12–9–55	61	30	Red stones. Hind limb of frogs. Larva of insect. Small fang. Red ants. Some parts of insects.	Small frog. Cestodes. Nematodes. Parts of insects. Frog bones. Stones. Half digested frog. Parts of beetle.	Big Nematodes. One beetle. One fang.	Sixteen echis fangs. Black beetle parts. Chitinous parts. Muscles parts. Plant parts. Eyes of an insect. Forficula parts. Entire beetle terga. Flower stalk. Earthy matter.

During 1954 it was possible to keep a large number of echis snakes alive by giving them tiny frogs. This was the first time, echis were kept alive for a period of more than ten months in this laboratory. It became difficult to get small frogs in summer, but now, after the above studies, insects would be used and the effect observed.

During these field trials the following significant observations have been made:—

(1) The area round Deogad is barren and rocky with small bushes and scattered number of stones. Snakes were found in summer and rains under these scattered stones, while during winter months they were sometimes recovered from between rocks.

	September-October 1954	February 1955	August-September, 1955
(2) The maximum temperature at Deogad	93°F.	93°F.	85·5°F.
The minimum temperature at Deogad	69°F.	65°F.	71·4°F.
The per cent humidity maximum at Deogad	98	90	98
The per cent humidity minimum at Deogad	63	43	81
Total rainfall at Deogad	12·04″	*Nil*	113″

(3) Forty-one common wild plants and 14 cultivated plants were collected while the staple crop in the locality was rice and nachni.

(4) More echis snakes of different sizes are available during and immediately after the rainy season.

(5) The snakes are actually recovered more when it is sunny than when it is raining.

(6) The maximum length of echis collected was 2 ft. 1 in. and this was during winter months. The minimum was four inches and was collected in rainy season.

(7) More snakes are actually collected (during the time of observation, i.e., 9 a.m. to 12 noon) under stones than in the open.

(8) The snakes collected in winter were lethargic and did not attack immediately, when cornered. But the snakes collected in summer were very ferocious and attacked with least provocation.

(9) The animals collected near such stones were insects, centipedes, millipedes, snails, scorpions, spiders, lizards, earthworms, frogs, field mice young-ones and occasionally other small snakes.

2. THE VENOM OUTPUT.

Last year the venom output of cobra, krait and Russell's viper snakes kept in the farm and in the room was studied. This was done by taking into account the average venom output of each snake during the period January 1954 to December 1954. These snakes were milked collectively. The statistical analysis of the data by applying the ' t ' test showed that the venom output was very significantly higher in case of cobra and Russell's viper snakes kept in farm than in the room.

From December 1954 to July 1955 a definite number of 13 snakes were regularly taken both from farm and room. They were both milked once a month and the venom of each individual snake recorded separately. The average venom output of these individual snakes kept in room and farm were subjected to ' t ' test of statistical analysis. It has been found from this analysis that the venom output of snakes kept in the farm, particularly cobra and krait, was significantly higher than similar snakes kept in the room. Table III gives these figures.

Russell's viper snakes bred in the farm during the month of May 1955.—The venom output of Russell's viper snakes significantly decreased a month before breeding, i.e., in May and continued to be so after breeding. Therefore, the average venom output of Russell's viper in farm as seen from the six months data was significantly lower than those kept in the room. There was no breeding in case of cobra and krait snakes kept in the farm and they have given much higher venom than in the room. These studies will be continued for six months more. It is also seen that the venom output of all snakes in the farm is maximum during summer and rainy months and minimum during winter. These individual venom output observations when completed in six months would be analysed statistically to confirm this second view.

It has been further observed that the snakes kept in the farm on an average live longer than snakes kept in cages. The mortality of snakes in the farm is maximum during breeding months, i.e., April to June, while the mortality of snakes in the room is spread all along. This data kept for individual snakes will also be completed within six months for statistical analysis confirmation.

TABLE III*a*.

Statistical analysis of venom output in gms. in snakes kept in the snake-farm as compared to those kept in the room.

(The averages are from a varying number of snakes from a common pool. Observations from January 1954 to December 1954. Only the significance of the farm yield for any snake has been mentioned with ' Yes ' or ' No '.)

	COBRA:		RUSSELL'S VIPER:		KRAIT:	
	Farm	Room	Farm	Room	Farm	Room
Grand mean of averages ...	0·165	0·122	0·134	0·106	0·0265	0·0266
Value of ' *t* ' ...	3·6735	...	2·856	...	1·102	...
Significance ...	' Yes '	...	' Yes '	...	' No '	...

TABLE III*b*.

The averages from a definite number of snakes. The snakes were milked individually. (Observations from December 1954 to July 1955).

Grand mean of averages ...	0·1589	0·1272	0·1025	0·1535	0·0286	0·0174
Value of ' *t* ' ...	2·043	...	...	5·07	4·73	...
Significance ...	' Yes '	...	' No '	...	' Yes '	...

3. Breeding habits.

It is seen in the snakes kept in farm and also brought from the field that the copulation takes place from July to September, but the breeding takes place next year from February onwards. It remained to be seen whether the sexual cycle in snakes consisted of courtship and copulation at one time and the laying of eggs many months afterwards. If the copulation had taken place in July to September, then one would expect the fertilization of the ova during this period followed by the development of the ova. In this case no spermatozoa would have been observed again prior to egg-laying, i.e., many months after it has been laid. The problem, therefore, is whether the development of the ova starts immediately after copulation or many months afterwards. In the latter case, where do these spermatozoa lie, and whether they are in hibernation. Non-poisonous snakes allied to cobra were taken for these studies. Observations have been done on *Coluber helena*, a non-poisonous snake, to see if there is any hibernation of spermatozoa in the female till ovulation takes place. For this reason six female snakes were dissected each month and their ovary, oviduct, cloaca and also pituitary fixed for sections. This is being done for the past twelve months along with the smear of the cloaca taken at the same time. The material is being studied in co-operation with the Indian Cancer Research Centre, Bombay. The studies are yet in progress. The snakes court and copulate during July to September months. The breeding takes place from March onwards. Smears of the cloaca have revealed spermatozoa during July to September and then they are seen only after February, probably prior to breeding. The question that remained was where does this spermatozoa lie in the female genitalia from September to February and whether this could come under the phenomenon of hibernation. If the sections revealed spermatozoa in the oviduct or thereby, in different months, then it would be possible to substantiate the above idea. The maximum activity of the ovarian cycle as seen by sections in proliferations will be correlated with the pituitary proliferations if any, to show if there is any correlation between them.

Conclusions.

Studies are being done to observe the food and other habits of echis snakes in the field with a view to keep these snakes for a long period in the laboratory and to prepare a hide out for them in the snake-farm on the basis of their habits observed in nature. The venom studies of cobra, krait and Russell's viper snakes will give us data to expect, apart from proving the greater output of venom in the snake-farm, varying amount from snakes in different months. It will also show that the snake-farm will give more venom as compared to snakes kept in the room, but if the snakes in the farm are to be used for breeding purpose then venom output may be less. This means that snakes in the farm could be either used for breeding or for keeping only. It will not be possible to have breeding and keeping at the same place and time in the same farm. The sections of the different organs will throw a light on the sexual cycle of snakes, thus giving a clue to keeping snakes at proper timing for venom output.

The morphological studies done last year remained to be finalized. When completed they would form an important contribution to the knowledge of structure of these animals so very valuable to us economically, particularly from medicinal point of view.

10. Inquiry into the changes, clinical and pathological, that are present months or years after apparent recovery from 'kwashiorkor' or 'nutritional dystrophy' under Dr. S. T. Achar at the Madras Medical College, Madras.

A. *Follow up of the 392 children admitted into the wards during 1949-1953 for nutritional œdema.*—Four more from this group were brought by the social worker after great difficulty, and were studied with regard to clinical condition and histologic changes in the liver. The main features in these children are noted below:—

Serial number	*Height	†Weight	Liver palpability	Serum protein	Albumin	Globulin	Thymol turbidity
34	H-1	W-2	1 finger	5·1	3·1	2	6
35	H-2	W-3	1 finger	5·2	2·5	2·7	6
36	H	W	Just palpable	6	3·8	2·2	5
37	H-1	W-1	1 finger	5·2	3·1	2·1	4

*H—Height (*minus* 1, 2, 3 degree of deviation from normal).
†W—Weight (*minus* 1, 2, 3 degree of deviation from normal).

There were no appreciable hair changes to be noted in any of the above. In one (case 35) there was angular stomatitis cheliosis and puffed out cheeks, but no parotid swelling.

Liver histology was studied in these four, as well as in the 33 cases previously reported. The changes noted in these 37 are classified as follows:—

(i) *Coarse periportal fibrosis of a slight degree.*—By this is meant that the main changes are in the portal tracts and consist of localized apparently quiescent slight fibrosis, with the limiting lamina intact and no invasion of parenchyma; this type of change was found in 14 of the 37 cases.

(ii) *Fine periportal fibrosis of a slight degree.*—Apparent increase of fibres having an irregular appearance in the periportal tract extending into parenchyma. This was found in 21 of the 37 cases studied. Two cases did not show any appreciable increase of fibrous tissue, coarse or fine, in the portal tracts.

(iii) *Centrilobular thickening of reticulum.*—This was found in a slight degree in three of the series but even so these changes were overshadowed by changes in the portal tract. It was not possible to locate the central vein in the great majority (28 out of 37) of these biopsy specimens.

(iv) *Thickening of reticulum in the lobules.*—This was the impression on going through these slides in almost every specimen—but this is only a tentative impression and has to be re-checked by comparison with biopsy specimens from apparently normal children—and this work is still in progress.

In this group there was one case which showed a little more advanced fibrosis on biopsy two years back (advanced in the sense that the periportal fibrosis tissue in some area was seen linking up with that round adjacent portal tract); but in a repeat biopsy recently there was hardly any of this linking and the periportal fibrous tissue seemed much less than previously.

Liver histology with regard to cellular infiltration and proliferation of Kupffer cells—our impression is that neither are prominent features in the great majority of slides studied.

Liver histology with regard to parenchymatous cells—all we can say is that the main feature noted was that the cells seemed to be taking the staining poorly in H. and E. specimens as also in the few cases in which slides were stained by toludin blue. Possibly, this signifies protein deficient cells. We have not found many cells with multinucleate nucleii and it is our impression that there is no evidence of great regeneration; neither did we find frank necrosis, though in some of the slides there were a few liver cells with abnormal staining-squashed cells with concave edges taking on an eosinophilic staining.

B. *Follow up of 33 children with past kwashiorkor who were reported last year.*—Eleven of these children were brought once again by the social worker for repeat study and in nine of them repeat liver biopsy was done. In two, the fine periportal fibrosis (mild) originally found was now not apparent but instead there was a condensation of this fibrous tissue into localized quiescent 'scars' (mild coarse periportal fibrosis). This makes us think that fine periportal fibrosis could condense into this type of quiescent scars. In one case which had rather more extensive coarse periportal fibrosis linking up with adjacent portal scars in the biopsy two years back, there was hardly any fibrosis visible now; two possible explanations occur to us, either the fibrosis was present but not visible in the lobules that could be got in the small bit removed at biopsy or that the fibrosis regressed.

In none of the specimens was there any appreciable increase of fibrous tissue. All the specimens except one showed nearly the same degree of pale staining of the parenchymal cells.

C. *Follow up of eight children previously reported as having hepatic fibrosis of a fairly extensive degree and hepatomegaly and evidences of nutritional deficiency with, however, no previous episode of kwashiorkor.*

Six of the children were followed over a period of six months to two years and three of them are still under observation, while the other three are lost sight of at the moment after months of follow-up—the interesting features are that all these six during their follow-up are running a benign course *without* development of portal hypertension or other complications of cirrhosis liver—all have showed fine periportal fibrosis of an extensive type originally and the three who are still being followed are showing condensation of the fibrous tissue into what look like extensive but quiescent scars.

D. *Study of three new cases of the above type encountered in recent months.*—They will be reported in due course after some more months of follow-up.

E. *Liver biopsy study (with reference to fibrous tissue and reticulum) of other children and infants coming to hospital with diverse conditions to serve as ' controls '.*—This study is still in progress.

11. Inquiry on treponemal agglutination tests for syphilis (TPA test) under Dr. C. W. Chacko in the Venereal Diseases Laboratory, Upgraded V. D. Department, Government General Hospital, Madras.

TPA-test in parallel with Wassermann, VDRL and Kahn test for syphilis on 810 specimens of sera has been done. This included 238 sera from known cases of syphilis in various stages, 170 sera from apparently normal persons and 290 sera from persons suffering from diseases other than syphilis. One hundred and twelve specimens had to be rejected for want of relevant data and/or inconclusive results obtained from them. The sensitivity of the TPA test in syphilis tends to be fair enough and comparable with the standard tests. The specificity of the test as checked in normal persons appears to be no better than the standard tests. The percentage reactivity of the test in diseases other than syphilis shows the same trend. Syphilis sera most probably contain two distinct agglutinating antibodies, one non-specific and the other specific in syphilis. It is possible that the potential specificity of the TPA-test would be manifested only when the test is performed on sera from which the non-specific antibody is absorbed previously. Adequate material and experimental data are not available at this stage to check and confirm this aspect so that no conclusion could be drawn yet.

Successful preliminary trials in the preparation of specifically agglutinable antigen suspension of the Nichol's strain of virulent *T. pallida* extracted from testes of rabbits in whom the strain had been established in serial subculture earlier had been carried out. A few trials of the TPA-test using this antigen on sera from known syphilitic and non-syphilitic persons had shown promising results earlier.

Source of the antigen.—Nichol's strain of virulent *T. pallidum* adapted to grow in live rabbit testes tissue.

The technique of preparation of the specific antigen and the TPA.—This test was essentially the same as described in the progress report of September 1954.

Technique of absorption of Wassermann antibody or reagin from serum.—Five volumes of 0·85 per cent NaCl solution were added to one volume of standard VDRL antigen. This antigen suspension was allowed to stand at room temperature for ten minutes and then centrifuged at 2,000 rpm for 30 minutes. The supernatant was decanted and precipitate resuspended in one volume of the NaCl solution. The serum to be absorbed was mixed with an equal volume of the re-suspended antigen and incubated at 37°C. for one hour and then placed in a refrigerator over night. The following morning the mixture was centrifuged at 2,000 rpm for thirty minutes. The supernatant was removed and is a one on two dilution of the original serum. Absorbed serum was tested with VDRL test and if it is still, positive absorption was repeated. Usually, the reagin disappeared after the first absorption. TPA-test is repeated quantitatively on the absorbed sera.

Results.

Categories of sera	Untreated	Treated	Total
Syphilis:			
Primary syphilis	12	5	17
Secondary syphilis	48	27	75
Latent syphilis	40	5	45
Late syphilis —benign ...	44	8	52
,, ,, CVS	7	2	9
,, ,, CNS	14	5	19
Congenital syphilis	16	2	18
Acquired syphilis in children ...	3	...	3
			238
Non-syphilitic venereal diseases:			
Lymphogranuloma venereum ...			16
Venereal granuloma			15
Gonorrhœa...			11
Chancroids			9
			51
Normals			170
Non-venereal abnormals:			
Yaws			4
Leprosy			109
Post-vaccination cases			20
Pregnancy			16
Diagnosis not established ...			7
Miscellaneous diseases ...			83
			239
Rejected as inconclusives			112
Total			810

TABLE I.

Primary syphilis—untreated.

Tests	Number of bloods tested	Positive	Doubtful	Negative	Per cent sensitivity	Remarks
TPA ...	12	11	0	1	91·7	Six positive sera were absorbed with VDRL antigen and were still positive with TPA-test and negative with VDRL test.
Wassermann ...	12	8	...	1	66·7	
Kahn ...	12	8	1	3	70·9	
VDRL ...	12	11	0	1	91·7	

TABLE II.

Primary syphilis—treated.

Tests	Number of bloods tested	Positive	Doubtful	Negative	Per cent sensitivity	Remarks
TPA	5	0	0	5	*Nil*	
Wassermann	5	4	0	1	80	
Kahn	5	0	0	5	*Nil*	
VDRL	5	1	0	4	20	

TABLE III.

Secondary syphilis—untreated.

TPA	48	48	0	0	100	
Wassermann	48	47	0	0	97·9	
Kahn	48	46	2	0	97·8	
VDRL	48	48	0	0	100	

TABLE IV.

Secondary syphilis—treated.

TPA	27	14	0	13	51·8	Five positive sera were still positive with TPA test and negative with VDRL test after absorption.
Wassermann	27	11	1	14	40·74	
Kahn	27	7	6	14	37·04	
VDRL	27	19	3	5	75·9	

TABLE V.

Latent syphilis—untreated.

TPA	40	40	0	0	100	Twenty positive sera continued to be positive with TPA-test and negative with VDRL after absorption.
Wassermann	40	37	0	3	92·5	
Kahn	40	36	3	1	93·75	
VDRL	40	40	0	0	100	

TABLE VI.

Latent syphilis—treated.

Tests	Number of bloods tested	Positive	Doubtful	Negative	Per cent sensitivity	Remarks
TPA ...	5	5	0	0	100	Three positive sera were still positive with TPA-test and negative with VDRL test after absorption.
Wassermann ...	5	4	0	0	80	
Kahn ...	5	4	0	1	80	
VDRL ...	5	5	0	0	100	

TABLE VII.

Late syphilis—benign untreated.

TPA ...	44	44	0	0	100	Twenty-three positive sera were still positive with TPA-test and negative with VDRL-test after absorption.
Wassermann ...	44	42	0	1	95·4	
Kahn ...	44	42	2	0	97·7	
VDRL ...	44	44	0	0	100	

TABLE VIII.

Late syphilis—benign treated.

TPA ...	8	7	0	1	87·5	Seven positive sera were still positive with TPA-test and negative with VDRL-test after absorption.
Wassermann ...	8	5	1	1	68·7	
Kahn ...	8	6	1	1	81·2	
VDRL ...	8	8	0	0	100	

TABLE IX.

Late syphilis—cardiovascular untreated.

TPA ...	7	6	0	1	85·7	Five positive sera were still positive with TPA-test and negative with VDRL-test after absorption.
Wassermann ...	7	7	0	0	100	
Kahn ...	7	7	0	0	100	
VDRL ...	7	7	0	0	100	

TABLE X.

Late syphilis—cardiovascular treated.

Tests	Number of bloods tested	Positive	Doubtful	Negative	Per cent sensitivity	Remarks
TPA ...	2	2	0	0	100	One positive serum was still positive with TPA-test and negative with VDRL-test after absorption.
Wassermann...	2	1	0	1	50	
Kahn ...	2	1	1	0	75	
VDRL ...	2	2	0	0	100	

TABLE XI.

Late syphilis—CNS—untreated.

TPA ...	14	14	0	0	100	Ten positive sera were still positive with TPA-test and negative with VDRL-test after absorption.
Wassermann...	14	14	0	0	100	
Kahn ...	14	12	2	0	92·8	
VDRL ...	14	14	0	0	100	

TABLE XII.

Late syphilis—CNS—treated.

TPA ...	5	5	0	0	100	One positive serum was still positive with TPA-test and negative with VDRL-test after absorption.
Wassermann...	5	5	0	0	100	
Kahn ...	5	5	0	0	100	
VDRL ...	5	5	0	0	100	

TABLE XIII.

Congenital syphilis—untreated.

TPA ...	16	15	0	1	93·7	Ten positive sera were still positive with TPA-test and negative with VDRL-test after absorption.
Wassermann...	16	16	0	0	100	
Kahn ...	16	12	3	1	84·3	
VDRL ...	16	16	0	0	100	

TABLE XIV.

Congenital syphilis—treated.

Tests	Number of bloods tested	Positive	Doubtful	Negative	Per cent sensitivity	Remarks
TPA ...	2	1	0	1	50	One positive serum was still positive with TPA-test and negative with VDRL-test after absorption.
Wassermann ...	2	0	0	2	*Nil*	
Kahn ...	2	0	0	2	*Nil*	
VDRL ...	2	1	0	1	50	

TABLE XV.

Acquired syphilis in children—untreated.

TPA ...	3	3	0	0	100	Three positive sera were still positive with TPA-test and negative with VDRL-test after absorption.
Wassermann ...	3	3	0	0	100	
Kahn ...	3	3	0	0	100	
VDRL ...	3	3	0	0	100	

TABLE XVI.

Lymphogranuloma venereum.

TPA ...	16	2	0	44	12·5
Wassermann ...	16	3	2	11	25
Kahn ...	16	1	2	13	12·5
VDRL ...	16	1	4	11	18·75

TABLE XVII.

Venereal granuloma.

TPA ...	15	10	0	5	66·66
Wassermann ...	15	3	1	10	23·33
Kahn ...	15	4	2	9	33·33
VDRL ...	15	7	1	7	50

Table XVIII.

Gonorrhœa.

Tests	Number of bloods tested	Positive	Doubtful	Negative	Per cent sensitivity	Remarks
TPA	11	3	0	8	27·28	
Wassermann	11	0	2	9	*Nil*	
Kahn	11	0	1	10	*Nil*	
VDRL	11	1	0	11	9·1	

Table XIX.

Chancroids.

TPA	9	0	0	9	*Nil*
Wassermann	9	1	0	8	11·11
Kahn	9	0	0	9	*Nil*
VDRL	9	0	0	9	*Nil*

Table XX.

Normals.

					Per cent specificity	
TPA	170	7	0	163	95·9	One serum which is positive with both VDRL and TPA became negative to both tests after absorption.
Wassermann	169	19	16	134	79·3	
Kahn	169	0	4	165	98·8	
VDRL	170	5	4	161	94·7	

Table XXI.

Yaws.

					Per cent reactors	
TPA	4	4	0	0	100	Four sera which were positive with both VDRL and TPA-tests continued to be positive with TPA-test but negative to VDRL-test on absorption.
Wassermann	4	2	0	2	50	
Kahn	4	3	0	1	75	
VDRL	4	4	0	0	100	

TABLE XXII.

Leprosy.

Tests	Number of bloods tested	Positive	Doubtful	Negative	Per cent Reactivity	Remarks
TPA	109	7	0	102	6·43	
Wassermann ...	109	31	5	73	30·74	
Kahn ...	109	8	3	98	8·72	
VDRL ...	109	10	5	94	11·47	

TABLE XXIII.

Post-vaccination.

TPA	20	4	0	16	20	
Wassermann ...	20	6	2	12	35	
Kahn ...	20	0	0	20	*Nil*	
VDRL ...	20	0	0	20	*Nil*	

TABLE XXIV.

Pregnancy.

TPA	16	0	0	16	*Nil*	
Wassermann ...	16	2	1	13	5·63	
Kahn ...	16	0	0	16	*Nil*	
VDRL ...	16	0	0	16	*Nil*	

TABLE XXV.

Diagnosis not established.

TPA	7	5	0	2	71·4	Two sera positive to both TPA and VDRL became negative with VDRL and continued to be positive with TPA-test on absorption. One serum positive to both tests became negative to both tests on absorption.
Wassermann ...	6	5	0	0	83·33	
Kahn ...	6	2	4	0	66·66	
VDRL ...	7	6	0	1	85·7	

TABLE XXVI.

Miscellaneous—non-venereal abnormals.

Tests	Number of bloods tested	Positive	Doubtful	Negative	Per cent Reactivity	Remarks
TPA	83	21	0	62	25·3	
Wassermann ...	83	29	10	44	34·9	
Kahn ...	83	9	10	64	16·9	
VDRL ...	83	20	2	16	25·3	

The specimens of sera of various categories for the evaluation of the TPA-test were obtained from patients of V.D. and other clinics of the General Hospital and the normals included medical staff and students. These specimens were stored in the cold and were tested at intervals with TPA-test Wassermann reaction, Kahn standard and VDRL slide-flocculation tests, qualitatively and quantitatively, in parallel.

Sensitivity of a test is expressed as percentage of positive reaction observed in known cases of syphilis. Doubtful reactions are given half the value of positive reactions in actual calculation.

Specificity of a test is expressed as a percentage of negative reaction observed in normal persons. Doubtful reactions are given half the value of negative reactions. In non-syphilitic diseases reactors are expressed as percentage of positive reactors similarly.

From the data displayed in the Tables, it would appear that the TPA-test has useful sensitivity comparable with the standard tests on 238 sera from known cases of syphilis in various stages.

As regards specificity from the results obtained in 170 cases of apparently normal persons, the TPA-test does not appear to show the expected complete specificity. The results are only just comparable with those obtained with standard tests. This seems to be the case in 290 non-syphilitic conditions too, as far as the reactivity percentage is concerned.

Earlier workers with TPA-test, namely Hardy and Nell (1954) and Mcleod and Stokes (1955) have indicated the presence of two treponema agglutinating antibodies in syphilitic serum. One is non-specific and may be identified with Wassermann antibody or reagin detected with standard tests for syphilis. The other is specific and is possibly identical with the immobilizing antibody of the treponema immobilization (TPI) test. It is possible to absorb the non-specific antibody with lipid antigen of standard tests leaving the other in the syphilitic sera to continue agglutinating the virulent treponema pallidum.

In this inquiry, 118 sera from known syphilitic persons which have been reactive to both VDRL and TPA tests have been absorbed with VDRL test lipid antigen, resulting in continued positive reaction with TPA test and negative reaction with VDRL-tests. This points out the possible presence of two distinct agglutinating antibodies in syphilis sera. If so, TPA-test done on sera after absorption with VDRL antigen would make its results completely specific in syphilis. Sufficient data and special categories of sera have not been available so far to confirm it. Only two sera, from non-syphilitic conditions which gave positive reaction to VDRL and TPA test, have been successfully absorbed with VDRL antigen to give negative reaction to both the tests indicating the absence of specific antibody. Efforts will be concentrated on false reacting non-syphilitic sera during the remaining part of the inquiry using the test-after-absorption-technique so that the specific value of the TPA-test in diagnosis of false reactions for syphilis with standard tests will be evaluated.

As regards the preparation of specifically agglutinable suspension of virulent *T. pallidum* from rabbit testes, the technique described has not proved consistently a satisfactory antigen, from every infected testes. It has not been easy to get the *T. pallidum* suspension rid of testis tissue debris completely, by differential centrifugation. Extraction and high speed centrifugation in the cold could not be managed due to lack of equipment for it. Under the circumstances, occasional non-specific clumping and a granular precipitate of rabbit testicular origin, not easily distinguished from similar granular materials originating apparently from disintegrated clumped *T. pallida* interfered with reading of test reaction and many inconclusive results. The amount of suspension of *T. pallidum* that could be extracted from each rabbit has been limited owing to the small size of testes of rabbits available. Cortisone-treated animals did not provide a better harvest of *T. pallida* in a few cases tried, as suggested by earlier workers. More extensive trials with greater dose of cortisone is indicated to see if a large harvest of *T. pallida* could be obtained from infected testes. A workable technique has, however, been established and it is hoped to conclude the inquiry on the TPA-test as planned.

12. Inquiry on anæmia and toxæmia as a result of amœbic infection under Dr. R. M. Kasliwal at the Sawai Man Singh Medical College, Jaipur.

(A) ANÆMIA AND TOXÆMIA IN CHRONIC AMŒBIASIS.

Anæmia.—In this series 60 cases were studied. The severity of anæmia varied from a mild to moderate degree. Only in very few cases, the red cell count was below 2·0 million/c.mm. The anæmia was of macrocytic hypochromic type. Sternal marrow examination showed a normoblastic reaction in a majority of the patients. Parenteral crude liver extract improved the anæmia in practically all cases. Iron preparations were, however, poorly tolerated by these patients. The cause of anæmia appears to be varied. Secondary infection of amœbic ulcerations with resulting toxæmia, mal-absorption, malnutrition and chronic minute hæmorrhages appear to be responsible for producing the anæmia of chronic amœbiasis.

Toxæmia.—A low degree of toxæmia has been seen in a majority of chronic amœbiasis cases. e.s.r. readings showed a slight increase in more than 90 per cent of the patients examined. These readings returned to normal or near normal values after a course of antibiotic and antiamœbic treatment. It is difficult to say as to how much of toxæmia is due primarily to the amœbæ or their products of disintegration and how much due to secondary bacterial associates. Since, however, a course of antibiotic *plus* antiamœbic drugs helps these patients in ameliorating the low grade toxæmic symptoms of malaise anorexia lassitude, etc. It is thought that both these factors operate in the production of toxæmia.

(B) HISTOPATHOLOGICAL LIVER CHANGES IN CASES OF COLONIC AMŒBIASIS —STUDY BASED ON EXAMINATION OF LIVER TISSUE OBTAINED BY NEEDLE BIOPSY OF LIVER.

In practically all the 60 cases of amœbiasis studied, the liver was slightly enlarged and tender on pressure or fist percussion of the lower right costal margin. Liver biopsy was done in 29 of these cases. The histopathological findings were as under:—

Reticulœndotheliosis of a varying degree was the commonest change seen in these cases. The other important change was diffuse parenchymal damage and disorganization of the hepatic cell plates. Focal necrosis and round cell infiltration of the portal tracts were present in a fewer number. Fibrosis and fatty infiltration was conspicuous by its absence excepting in a single liver biopsy specimen. Central veins were normal and no thrombosis was seen in any of the vessels.

Invasion of the human body by *Entamœba histolytica* produces a bewildering variety of symptoms and signs. The diversity of complaints can be judged from the fact that Boyers (1933) listed 1961 separate complaints from 700 patients suffering from this infection. Besides the usual complaints referred to the gastro-intestinal tract, nervous system and cardiovascular system, etc., we had observed that many of our patients suffered from varying degrees of anæmia. Such an observation has been previously made by some Western authors, but as far as we have been able to find out, except for the study of Hinman and Kampmeier (1931) no detailed study of anæmia in amœbiasis has been made although isolated references to the occurrence of a secondary anæmia in chronic amœbic patients have been encountered. Thus, Craig (1944) mentions that among the physical signs of chronic amœbiasis a mild secondary anæmia may be present. Shrivastava (1951) who carried out a human survey of intestinal parasitic infections at Bombay, found that anæmia was present in slightly less than ten per cent of the persons having *Entamœba histolytica* infection. Anderson *et al.* (1953) have cited Snell (1946) having found anæmia as one of the clinical manifestations of amœbiasis. Whitby (1946) mentions amœbic dysentery as a cause of anæmia in diseases of the alimentary tract. Kasliwal (1954) in a survey of hospital admission for amœbiasis found anæmia an important feature. Of course, the occurrence of anæmia in cases of fulminant tropical liver abscess is well known.

Although it has been clinically observed that many cases of chronic amœbiasis present a toxic look and symptoms suggestive of low grade toxæmia, such as malaise headache, a pale worn look, etc., no objective studies have been made to demonstrate the presence of toxæmia in amœbiasis. We have in our study taken routine Erythrocyte sedimentation rate readings and have attempted to confirm this clinical observation.

METHODS AND MATERIALS.

A total of 60 patients was studied in this series. The diagnosis of amœbiasis was confirmed in all cases by examination of stools for

Entamœba histolytica. In general, three routine examinations in saline and iodine were done and another stool examination, a fourth examination was made in those cases whose stools were negative, but who presented the clinical picture of amœbiasis, after giving magnesium sulphate early morning and thereafter examining the stools for the parasite. Zinc sulphate centrifugal floatation concentration technique of Faust *et al.* (1939) was also used whenever such supplementation was deemed necessary.

After the *Ent. histolytica* had been found in the stool, blood examination of the patient was done. This consisted in doing the following investigations:—

1 Total red blood cell count.
2 Hæmoglobin estimation (g. per 100 g.)
3 Erythrocyte sedimentation rate (Westergren).
4 Packed cell volume.
5 Absolute values.

Besides these, the sternal bone marrow examination was done in 28 patients. The smears were examined for the presence of erythroblastic hyperplasia and the predominant cell type.

(1) *Total red blood cell count.*

A standard r.b.c. diluting pipette was used. Gower's fluid was used as the diluting fluid (sodium sulphate Crystalline 4·5 gms., Glacial acetic acid 1·5 c.c., distilled water to make 100 c.c.). Counting of the cells was done in Spencer chamber with improved Neubauer ruling. The red blood cells in five sets of sixteen small squares were counted diagonally across from one end to the other.

(2) *Hæmoglobin estimations.*

Hellige hæmoglobinometer (in which the colour of acid hæmation of the examined blood is compared with standard tinted glass) was used. During the course of this study, the Hæmoglobinometer was occasionally matched with a standard and corrections made whenever necessary.

(3) *Erythrocyte sedimentation rate.*

Westergren's technique was used here. The estimations were carried out at room temperature.

(4) *Packed cell volume.*

The Wintrobe p.c.v. tubes were spun in the centrifuge at 3,200 r.p.m. and the p.c.v. was determined at the end of 30 minutes. Another reading was taken at 40 minutes. If these two consecutive readings coincided the second reading was taken further at ten minutes interval till two consecutive readings were identical.

(5) *Absolute values.*

(*i*) Mean corpuscular volume :—(M.W.)

$$\frac{\text{Packed cell volume per cent} \times 10}{\text{R.b.c. in million per c.mm.}}$$

(*ii*) Mean corpuscular hæmoglobin concentration :—(M.C.H.C.)

$$\frac{\text{Hb. per cent g. per 100 c.c. of blood} \times 100}{\text{Packed cell volume per cent.}}$$

Normal values (Wintrobe, 1932).
Mean corpuscular volume—80 to 94 mm.
Mean corpuscular hæmoglobin conct.—32 to 38 per cent.

STERNAL MARROW EXAMINATIONS.

The smears were stained with Leishman's stain. A qualitative appraisal was made of the film to find out whether or not there was any hyperplasia of the erythropœtic tissue and to find the predominant cell type. The terminology used for the various types of normoblasts was normoblasts 'a', 'b' or 'c'. The generally used equivalents of these terms are, respectively, early normoblasts, intermediate normoblast and late normoblasts. In a few cases the sternal marrow smears were stained by Prussian blue to determine the relative amount of intracellular and extra-cellular iron. This examination was later on abandoned because of poor results obtained.

These hæmatological investigations were made before the start of any treatment after the patient had been shown to be infected with *Entamœba histolytica*. The investigations were repeated after giving a standardized course of treatment consisting of the following:—

(1) Crystalline penicillin G., 50,000 units intramuscular every four hours (four days).

(2) Emetine hydrochloride gr. 1 Intramuscular by daily (four days).
In the early part of the study these two drugs were given consecutively, but later on in order to shorten the hospital stay of the patients, these were given simultaneously.

(3) This was followed by a ten day treatment with Carborsome, 0·25 g. being given twice daily. During the whole course no hæmatinics were given.

The hæmatological status was evaluated at the end of this treatment. Those patients who still showed anæmia were given hæmatinics. The two drugs used were; (*i*) Crude liver extract 2 c.c. intramuscularly daily for ten days and (*ii*) Ferrous gluconate, three tablets daily for a similar period (·06 g. daily). The patients were given either of the two drugs. Since it was found that patients responded better to liver extract than to iron, a comparatively much larger number of patients in this series were those who received liver extract.

The third and final hæmatological study was made after this course of hæmatinics was completed.

RESULTS.

Anæmia:—

TABLE I.
(*Presence of anæmia.*)

Total number of cases studied ... 60

	Number	Percentage
Cases showing normal blood count (above 4·5 mill./c.mm.)	8	13·33
With anæmia	52	86·67

TABLE II.

(Degree of anæmia.)

Total number of cases studied ... 52

	Number	Percentage
Mild anæmia (3·5 to 4·5 mill/c.mm.)	28	53·85
Moderate anæmia (2·0 to 3·5 mill./c.mm.) ...	20	38·46
Severe anæmia (below 2·0 mill./c.mm.)	4	7·69

TABLE III.

(Type of anæmia.)

(A) *According to size (mean corpuscular volume) :—*

	Number	Percentage
Macrocytic	27	51·92
Microcytic	4	7·69
Normocytic	21	40·39
(B) *According to hæmoglobin conct. (M.C.H.C.) :—*		
Hypochromic	38	73·08
Normochromic	14	26·92
Normal values (Wintrobe, 1932)		
M.c.v.	80 lb. to 94 lb.	
M.c.h.c.	32 to 38 per cent	

TABLE IV.

(Sternal marrow examination.)

Total number of cases 71

		Number	Percentage
(*a*)	Hyperplastic marrow	5	23·80
	Normal marrow	16	76·20
	Megaloblastic marrow	1 (?)	4·70
(*b*)	Normoblastic ' a ' or ' a' and ' b ' ...	8	38·20
	Normoblastic ' c '	12	76·20

Constitutional symptoms attributable to toxæmia of chronic infection were seen in all the patients as shown in Table V :—

TABLE V.

Total number of cases 60

Symptoms	Number of cases	Percentage
(1) Æsthenia	46	76·66
(2) Loss of appetite	53	88·33
(3) Vague body pains	25	41·66
(4) Bad taste in the mouth	16	26·66
(5) Headache	14	23·33
(6) Low fever	9	15·00

The most common symptoms were loss of appetite and æsthenia.

E.s.r. readings were taken in 52 cases at the start of hospital treatment. Forty-nine of these patients showed readings above 10 mill./ at the end of one hour by Westergren's technique. After a course of antibiotic and antiamœbic treatment the E.s.r. readings came down to normal or nearly norm values.

TABLE VI.

(*Erythrocyte sedimentation rate*).

Number of cases 52

E.S.R.	Number of cases	Percentage
10 mm./1 hour or above	49	92·86
Below 10 mm./1 hour	3	7·14

Thirty-eight patients were given hæmatinics drugs after a preliminary course of antibiotic and antiamœbic therapy. The patients were placed in two groups (*i*) those receiving parenteral crude liver extract 2 c.c. I.M. daily for ten days —31 patients; (*ii*) those receiving ferrous gluconate. ·06 g. daily in divided doses—7 patients. The results obtained are shown in Table VII.

TABLE VII.

(*Response to hæmatinics.*)

Number of cases 38

BEFORE TREATMENT:			AFTER TREATMENT:	
Name	Hb. per cent g.	R.b.c. mill.	Hb. per cent Gm.	R.b.c. mill.
(A) *Liver extract—crude—parenteral—*				
1. U. ...	11·0	3·75	12·5	4·25
2. M. ...	10·5	4·05	12·5	4·80
3. M.C. ...	10·75	3·75	11·75	4·25
*4. M. ...	7·5	3·02	10·0	3·45
5. J.R. ...	9·0	3·25	11·0	4·15
*6. R.N. ...	11·5	3·0	12·75	3·75
7. M.K. ...	11·25	3·63	12·50	4·19
*8. G. ...	6·5	2·60	8·0	3·15
9. F. ...	10·0	3·0	12·5	4·1
10. N.L. ...	11·0	3·8	11·25	4·1
11. B. ...	10·0	2·85	11·5	3·30
12. N.L. ...	11·5	3·17	12·5	4·1
13. L.R. ...	12·5	4·1	14·0	4·6
14. L.C. ...	10·0	2·84	12·0	3·50
–15. R.N. ...	9·0	3·5	10·0	3·75
16. G. ...	4·0	1·75	7·0	2·01
*17. H. ...	9·5	2·92	10·5	3·20
18. J.K. ...	12·0	4·45	12·5	4·60
19. K. ...	13·0	3·80	14·0	4·10
20. C. ...	9·0	3·20	10·5	3·70
21. M. ...	10·5	4·05	11·75	4·75
*22. B.B. ...	6·75	2·20	8·0	2·75
23. K.K. ...	10·75	3·90	12·50	4.70
24. J. ...	11·0	3·60	12·0	4·09
25. M. ...	11·0	3·75	11·75	4·09
26. B. ...	10·0	3·75	11·5	4·15
27. V.L. ...	10·5	3·5	12·25	4·40
28. N. ...	5·25	1·70	9·25	2·05
29. H.S. ...	10·3	4·45	12·3	4·80
*30. B.S. ...	11·75	4·45	12·3	4·80
31. M. ...	9·0	2·90	11·0	3·75
(B) *Ferrous gluconate:—*				
†1. N.L. ...	5·5	2·08	6·0	2·19
2. N.R. ...	11·5	4·18	12·0	4·25
3. P. ...	12·5	3·91	12·75	4·05
4. K.R. ...	10·75	4·45	12·50	4·70
†5. B. ...	12·75	4·50	13·0	4·8
6. R.P. ...	12·25	4·15	13·0	4·63
†7. H.N. ...	11·0	3·95	11·75	4·15

*Patient left hospital before completing treatment.

†Patient had symptoms of iron intolerance.

DISCUSSION.

In the 60 cases of chronic colonic amœbiasis, anæmia of varying degree was present in 52. In a majority (53 to 85 per cent) the anæmia was macrocytic. M.c.h.c. was below the normal range in 38 (73·08 per cent) patients.

Sternal marrow examination was done in 21 cases. Twenty patients showed a normoblastic reaction. Marked normoblastic hyperplasia was present in five cases. The other 16 cases did not have hyperplasia of the erythropoietic tissue. Normoblasts (c) were predominant in 12 cases and Normoblasts ‘ a ’ or ‘ a ’ and ‘ b ’ in the other eight patients. One patient showed a doubtful megaloblastic reaction. Thirty-eight cases were given hæmatinic drugs, 31 being given parenteral liver extract and the others Ferrous gluconate. The cases treated with parenteral crude liver extract showed improvement of their anæmia. Iron did not have much value however, as a hæmatinic patients who received Iron showed a comparatively poor hæmatological response and in three patients there were gastro-intestinal upsets due to iron intolerance. Erythrocyte sedimentation rate readings which were used as a measure of toxæmia, showed in more than 90 per cent cases values of 10 mm. per hour or above. These values returned to normal or near normal levels after a course of antibiotic and antiamœbic drugs, alongwith the amelioration of symptoms of low grade toxæmia.

It was apparent thus that anæmia and toxæmia were present in cases of chronic amœbiasis. The severity of anæmia varied from mild to moderate and infrequently the red blood cell counts were below 2·0 mill./cmm. The causes of the anæmia, we think, were varied. Hæmorrhage, secondary infection of the amœbic ulcers with toxæmia, malabsorption and malnutrition were some of the contributory factors. A similar opinion has been advanced by Bankers (1951) who states that slight but constant blood loss from the bowels, toxæmia, and anorexia are the factors responsible for anæmia of amœbiasis. It is difficult to evaluate, however, the relative part played by toxæmia and deficiency of hæmatinic factors in production of anæmia in amœbiasis responsible for anæmia. In most cases probably both factors are operating.

The raised e.s.r. readings in our cases suggested the presence of toxæmia. This correlated well with the clinical evidence of low grade toxæmia in chronic patients of amœbiasis. The cause of this toxæmia was debatable. It may be that the *End. histolytica* itself produced a toxin or that the toxæmia was due to secondary bacterial associates infecting the amœbic ulcers.

SUMMARY.

Sixty cases of chronic amœbiasis were examined for the presence of anæmia and toxæmia. Fifty-two of these patients showed a varying degree of anæmia and toxæmia (as judged by e.s.r. readings clinical symptoms). The anæmia was dimorphic in a majority of cases. Treatment with an antibiotic, antiamœbic and hæmatinic drugs caused recovery in most cases. Parenteral crude liver extract had a definitely better value as a hæmatinic as compared to Iron. E.s.r. values and clincial symptoms improved after treatment.

LIVER CHANGES IN COLONIC AMŒBIASIS.

Liver involvement in amœbiasis in the form of amœbic hepatitis and amœbic liver abscess has been known for a long time. Loeber and D'Antoni (1947), while studying amœbiasis in children, reported finding liver enlargement and tenderness rather frequently in those having colonic amœbiasis. Such hepatomegaly occurs without any systemic reaction, such as fever, leucocytosis, etc., and the liver tends to return back to normal size after use of antiamœbic drugs. Sodeman (1950) has reported similar findings in adult patients. Recently, Kasliwal (1954) in a survey of hospital patients of amœbiasis pointed out that hepatomegaly in association with colonic tenderness was a very common finding in such patients. No histopathological study of such livers has yet been reported. We have done liver biopsy in cases of colonic amœbiasis coming under our care and the histopathological findings are reported below.

METHODS AND MATERIALS.

Twenty-nine cases of amœbiasis proved positive for *Ent. histolytica* by stool examination were studied. Liver biopsy was done using a Vim Silverman needle and the intercostal approach. Sections were stained by hæmotoxylin and eosin.

RESULTS.

The normal architectural pattern of the liver lobule was generally maintained, although in 24 cases the hepatic cell plates were disorganized. All cases with the exception of three showed some degree of diffuse parenchymal damage. This consisted of obscured cellular outlines, rarefaction or fragmentation of the cytoplasmic contents, eosinophilic smudging, variations of nuclear size in the same zone of the hepatic lobule and pyknosis or ballooning of the nuclei. Some patches of cells were anuclear. In addition, areas of focal necrosis provided another frequent change, being present in 21 cases. This consisted of dissolution of patch of parenchymal cells which were overrun by motley of infiltrating cells including polymorphs, mononuclear cells and histiocytes. These foci had no specified distribution in the lobule and varied in severity from case to case. Sometimes only a single such small area was seen in the entire tissue; in others, several such wide patches were seen. The trophozoites of *E. histolytica* were looked for and could not be identified with certainty.

Reticuloendotheliosis was the commonest change, being present in 27 cases. This also varied in severity. The milder change consisted of simply a swelling of the endothelial cells lining the sinusoids, which, bulged into the lumina. In severe cases the cells had undergone active proliferation and clumps of almost 5 to 20 cells with hyperchromatic nuclei distended the sinusoids. Only rarely these cells contained a light brown pigment.

Portal infiltration was seen in 12 cases. The infiltrating cells were mainly mononuclears, although a few polymorphs were also included. This change, however, was never very severe.

Fibrosis-portal or intralobular was conspicuous by its absence in all cases but one. In this case the fibrosis was limited to the portal area which was considerably enlarged and included two to three proliferating biliary ductules. The fibrosis did not extend into the lobule and the normal lobular relationship were still maintained.

Regeneration of the liver cells was a variable feature, seen only to a slight degree in some livers. Central veins were invariably normal. No thrombosis was seen in any of the vessels.

Only two specimens showed an insignificant amount of fatty change, limited to a small group of cells.

DISCUSSION.

Hepatic amœbiasis thus consists of colonic amœbiasis with associated hepatomegaly (?) (pre-amœbic hepatitis stage), amœbic hepatitis and amœbic liver abscess. Hepatic enlargement in colonic amœbiasis occurs in a singificant number of cases. This state represents an individual on his way to developing acute amœbic hepatitis or amœbic liver abscess. It has been suggested that this change in the liver is needed by the Entamœbe for a satisfactory implantation in sufficient quantities to produce an acute amœbic hepatitis or the further advanced stage of abscess (Sodeman, 1950). Histopathological changes in such livers show a picture of subacute hepatitis. The cause of this change may be amœba, its products of disintegration or the secondary bacterial associates of the amœbic ulcers. Such changes are in great probability reversible, as the hepatomegaly diminishes markedly following treatment with antiamœbic drugs either acting on the tissue face or on the intestinal amœbæ. The recognition of this stage is important because it means that the patient can be treated at a time when no drugs giving serious reactions need be given; they can be treated as ambulatory patients and the development of advanced states of hepatic amœbiasis with no insignificant mortality can be prevented.

SUMMARY.

Histopathological study by liver biopsy of 29 cases of hepatomegaly associated with colonic amœbiasis was made. The changes found were; reticulœndotheliosis, diffuse parenchymal damage, disorganization of the hepatic cell plates, focal necrosis of varying degree and round cell infiltration of the portal tracts. Fibrosis and fatty infiltration were minimal being seen only in two cases. These changes are probably reversible. *E. histolytica*, its products of disintegration or bactæria associated with the amœbic ulcers are thought to be the cause of this subacute hepatitis. Recognition of the disease at this stage would prevent further progress to well marked hepatitis or liver abscess.

13. Inquiry into the use of artificial hypothermia (hibernation) in open intracardiac surgery under Dr. P. K. Sen at the Seth G. S. Medical College, Bombay.

Seventy experiments have been carried out since 1st October 1954. The work has been divided under four main heads:—

(*a*) Certain groups of controlled experiments to study the causation, methods of prevention and treatment of ventricular fibrillation occurring during low temperature cardiotomies totalling forty.

(*b*) Surgical procedures to develop surgical technique—seven experiments.

(*c*) Studies on the isolated heart-lung preparation with intact coronary and pulmonary circulation to increase the survival time of such preparations with a view to

(*d*) Total heart-lung transfer from one frozen animal to another and study of certain drugs, e.g., prostigmine, potassium, etc., on the isolated heart, 12 in number.

Eleven experiments are not included in any of these groups as some errors crept in the course of the experiments, such as failure of electricity, or clamps cutting through vessels, etc.

(*a*) CONTROLLED EXPERIMENTS.

It was found from our previous experiments that ventricular fibrillation accounted for about 2/3rd of the total mortalities as reported in the previous report. After perfecting a standard method of production of ventricular fibrillation the present series of experiments were conducted to determine the effect of various drugs and ventilation in the prevention of artificially created ventricular fibrillation under artificial hibernation.

The method used is as follows: Adult mongrel dogs of 11 kg. to 15 kg., are subjected to artificial hibernation after intravenous pentothal and intubation by immersion in a tub containing pieces of ice floating. When the rectal temperature is 26°C. they are taken up on the table and hooked to the E.C.G. machine and constant reading taken as the thorax is opened and superior and inferior venacavæ are isolated by dissection and passing twine under them. Clamps are applied to these vessels and then aorta is clamped. Then the drug to be tested is injected into the left ventricle and a standard time duration of four minutes is allowed in all cases for the drug to perfuse through the coronary system. At the end of four minutes ventricular fibrillation is created by injection of 3 c.c. of 1:20,000 adrenaline. The E.C.G. records are studied for the reaction of the heart.

These experiments have been classed under ten groups:

1. Normal ventilation with air.
2. Normal ventilation with pure oxygen.
3. Hyperventilation with air (48 times a minute).
4. Hyperventilation with intravenous KCl.
5. Hyperventilation without surgery and without I. V. fluids.
6. Normal ventilation with intravenous KCl.
7. Hyperventilation with glucose and KCl intravenous.
8. Hyperventilation with only glucose.
9. Hyperventilation with intracardiac KCl of various percentages into the left ventricle after clamping outlet.
10. Hyperventilation with intracardiac prostigmine 1:4,000 2 c.c.

Table I gives the breakdown of these experiments.

TABLE I.

Serial number	Type of variations	Total number of experiments	Ventricular fibrillation developed in	Resuscitatory measures adopted			Reversion to normal rhythm	Remarks
				Cardiac massage	Electric shock defibrillation	Intracardiac KCl. 1·2 per cent		
1	Normal ventilation with air	2	2	2	...	...	...	...
2	Normal ventilation with 100 per cent O_2 ...	5	3	3	...	3 (10 c.c.)	1	KCl and massage
3	Hyperventilation with air	5	2	2	...	2	...	...
4	Hyperventilation and KCl I.V. ...	5	3	3	1	...	2	Only massage
5	Hyperventilation without surgery or I.V. fluid	5	Group used for biochemical studies.					
	Groups of experiments carried out during the present period.							
6	Normal ventilation with I.V. KCl ...	2	2	2	2	...	...	...
7	Hyperventilation, glucose and KCl ...	5	4	4	...	...	4	3 with massage 1 spontaneous
8	Hyperventilation and only glucose ...	5	3	2	...	2	3	KCl 1 and massage 2
9	Hyperventilation and 2 c.c. 1:4000 prostigmine	9	3	3	...	...	3	2 on repeat dose of 2 c.c. prostigmine
10	KCl into L.V. ...							
	(*i*) 0·1 per cent 10 c.c.	5	...	...	...	...	...	...
	(*ii*) 1 per cent 1 to 2 c.c.	10	1	1	...	...	...	Massage, no effect
	(*iii*) 2 per cent 1 to 2 c.c.	5	2	2	...	...	...	Massage not effective
	(*iv*) 1·2 per cent 1 to 2 c.c.	8	2	1	...	...	1	Without massage did not return to normal.

(*b*) SURGICAL PROCEDURES TO DEVELOP SURGICAL TECHNIQUES.

The second group of experiments has been concerned with the development of surgical techniques. The total number of experiments done is seven. Here we have endeavoured to produce certain cardiac defects as well as to plan and execute various intracardiac procedures.

The surgical procedures so far attempted are:—

1. Mitral instrumentation after clamping pulmonary artery (one experiment).
2. Creation and closure of auricular septal defect (two experiments).
3. Creation of ventricular septal defect (three experiments).
4. Finding out possibility of working under water (one experiment).

It is found among other things that it is possible to open the left side of the heart (left atrium) after clamping the main pulmonary artery for about four minutes without producing acute corpulmonale. This line of investigation is being pursued at present.

(*c*) STUDY ON THE ISOLATED HEART-LUNG PREPARATION.

The problem of transferring the whole heart with the lungs from one animal to another presents many problems of great complexity of which only one is the cessation of circulation that must follow during such a transfer. Our researches with hypothermia seemed to provide an answer to this difficulty as in the state of hibernation the brain can withstand stoppage of circulation for much longer periods than at normal temperatures. It was argued, therefore, by us that if such a transfer were to take place from one cooled animal to another and the time of such a transfer did not exceed the average time of safety it may be possible to obtain such a transfer without producing any cerebral damage. Accordingly, the following 13 experiments were planned and executed.

METHODS AND MATERIALS.

First stage: This consisted of removing an intact heart-lung preparation whose circulation and economy were inter-dependent and were together self-supporting as it were. Adult mongrel dogs were used throughout. Anæsthesia is induced with intravenous pentothal and the animal is put under positive pressure hyperventilation (48 per minute) through an intratracheal tube. The animal is then cooled to 27° C. in a tub of water with pieces of ice floating in it and then strapped on the table in the supine position. A transverse incision across the fifth interspace and sectioning the sternum transversely at that level after ligating the internal mammary gives an excellent approach to the thorax. A rib spreader is introduced and dissection commenced in the superior mediastinum. The superior venacava is freed, the pericardium is opened and the ascending aorta dissected from the anterior pad of fat which hides most of the aorta. The inferior venacava is dissected and a loop passed under. The cardiac lobes of the lungs are freed from the diaphragm and the plane of separation extended to the mediastinum behind the heart. Then the heart is rotated forward exposing the bronchial arteries which are clamped, ligated and divided. The azygos vein is likewise dealt with. Now the stage is set for separating the heart-lung preparation.

With a time-keeper calling out the minutes the separation is begun. The venacavæ are ligated and divided, a clamp is placed across the terminal part of the ascending portion of the aorta thus blocking the left ventricular outflow. One c.c. of KCl., 1·2 per cent is injected into the left ventricle for its effective perfusion through coronary circulation and so prevention of the V.F. The aorta is then divided and by lifting up the heart-lung unit it is possible to dissect in the fascial planes and divide the nerves and detach the entire specimen by severing the trachea. Another intratracheal tube is passed and the heart lung is placed on a towel and the reaction taken by a direct cardiac electrode. Table II gives the protocol of behaviour of the specimen:—

TABLE II.

Protocol of behaviour of the isolated heart lung.

Number	Total period of survival, minutes	Period of rhythmic beats, minutes	Period of effective beats, minutes	RATE OF BEATS	
				Maximum	Minimum
238	9	8	4	100	7
240	35	22	18	100	27
247	25	20	18	90	15
248	11	7	5	65	10
249	27	18	13	75	10
250	32	32	7½	100	15
251	33	33	23	60	15
252	21	19½	9½	100	20
254	25	14	10	75	15
261	30	25	25	100	16
263	25	20	20	85	20
265	30	30	25	90	20

(*d*) BEHAVIOUR OF THE ISOLATED HEART-LUNG PREPARATION.

The ventilation remained more than adequate as the specimen remained pink including the heart and coronary arteries. As will be seen from Table III period of survival varied from nine minutes to 35, but the period of effective beats continued for an average of ten minutes. In one instance effective beats continued for 23 minutes, and in two dogs up to 18 minutes. This was mainly obtained through the agency of drugs, such as calcium digitalis and adrenaline. The effects of the drugs are set out in Table III.

Since electrocardiograms were taken by direct cardiac leads some evidence of injury pattern was seen in almost all tracings, but occasionally this was exaggerated greatly with marked elevation of the ST segment. Since inversion of the T wave is a usual accompaniment of deep hypothermia the T wave changes could not be regarded as significant, but in most specimens the T wave which had become upright after injection of potassium in the left ventricle prior to removal of the specimen became gradually flattened and ultimately inverted as the beats in the isolated specimen became weaker and heart grew more flabby.

TABLE III.

Effects of drugs on the isolated specimen.

Number	Medication and particulars
238	(1) Calcium gluconate improved beats and (2) adrenaline converted feeble beats into fibrillation.
240	(1) 1·2 per cent KCl and calcium gluconate and 1 c.c. acetylcholine injections brought back the rhythm of the fibrillating heart but it again went into ventricular fibrillation. Then calcium gluconate or digoxin had no effect.
247	(1) Digoxin 0·125 had no effect on the size of the heart but contractions improved, (2) calcium gluconate improved the contractions, (3) adrenaline produced tachycardia and ventricular fibrillation, (4) prostigmine reverted back ventricular fibrillation to ventricular rhythm but it again went into ventricular fibrillation.
248	(1) Ventricular fibrillation noted during removal. KCl. prostigmine and massage had no effect. Again digoxin, calcium, massage and adrenaline 1 c.c. brought back normal rhythm.
249	(1) Calcium gluconate made beats stronger, (2) adrenaline turned feeble beats into flutter and ventricular fibrillation.
250	(1) Calcium gluconate and adrenaline had no marked effect on feeble contractions.
251	(1) After five minutes beats became feeble. Adrenaline 1 c.c. made beats stronger. It was beating powerfully till end when it gradually stopped.
252	(1) Digoxin had no effect on feeble contractions. Calcium gluconate made powerful contractions. Prostigmine had no effect on ventricular flutter.
254	(1) Calcium gluconate made feeble contractions powerful, (2) adrenaline turned feeble contractions into fibrillation, (3) prostigmine reverted back fibrillation to ventricular rhythm but it again went into ventricular fibrillation.
261	(1) Calcium gluconate made beats powerful. Adrenaline and digitalis also made beats powerful.

The study of electrocardiographic changes has formed an important part of our inquiry and continuous E.C.G. records are taken in both the controlled series and these experiments.

14. Effect of metaphyseal stimulation of longitudinal bone growth under Dr. B. Mukopadhya at the Prince of Wales Medical College, Patna.

Young goats, three to four weeks old, have been used as experimental animals. Autogenous cortical bone grafts have been inserted in the metaphyseal regions of the growing ends of the femur and the tibia with a view to stimulate the activity of the epiphyseal growth plates. Only one limb has been submitted to grafting operation in each animal using the contra-lateral opposite limb as control. The following conclusions have been drawn:—

(*a*) The grafting technique gives rise to a temporary and short-lived stimulation of growth, both longitudinal and circumferential. The growth stimulation is not due to mere operative trauma.

(*b*) The technique used does not damage or destroy the growth plates.

(*c*) The ultimate length of the bone achieved at the end of the period of growth is not influenced by this type of experiment. Once the stimulus ceases to act, the pre-determined length of the bone based on hereditary, congenital, metabolic and other factors serves to assert itself and the bones of the two sides get equalized in length. Some increase in circumferential diameter, however, persists even after growth ceases.

(*d*) Insertion of bone grafts is accompanied by an increase in the vascularity of the local area but this vascular reaction also subsides rapidly as the graft gets absorbed.

In last year's note reasons were advanced for the selection of goats as experimental animals. Male goats were castrated when they reached the age of eight to ten weeks. While reviewing last year's report, the Clinical Advisory Committee noted that ' In view of the marked influence of sex hormones on endochondral bone formation the Committee hoped that you have taken note of the sex hormone imbalance which you create through castration in the planning of your experiments.' Castration was performed after careful thought. It is well-established that castration delays the fusion of the epiphysis with the diaphysis of long bones. As a result, endo-chondral bone formation at the epiphyseo-metaphyseal junction continues for a relatively longer period in castrated animals as compared to uncastrated animals. The present series of experiments are not designed to study the general growth properties and growth potentialities of the epiphyseal growth plates. They are designed to study the effects of a non-damaging stimulus—a stimulus which causes an increase in the vascularity of the region of the metaphysis—applied to the vicinity of the epiphyseal growth plates. The results of the experiments depend entirely on the local tissue reaction caused by this stimulus. The general influences that determine endo-chondral bone formation act equally on all the epiphyseal cartilages of the animals experimented upon. Thus, the effects of castration act equally on the operated and the contra-lateral un-operated normal limb. In order to meet the objections of the Committee, the experiments were repeated on a further series of six male goats, all of whom have been left uncastrated. Since the basis of assessment in this series of experiments is the comparison between the operated and the contra-lateral un-operated limb of the same animal, no control series of castrated but ungrafted animals have been observed.

A total of 44 animals has so far been operated upon. Of these six animals have been lost, two in studying the local anatomy of the long bones and four from post-operative wound infection. Initially, animals were shaved two days before operation and the skin prepared as for elective operations in human patients. But it was soon realized that such pre-operative preparation in animals increase the rate of wound infection. It is impossible to keep the animals clean during the two pre-operative days.

The dressings become dirty and the exposed and rather raw skin becomes heavily contaminated. The coat of fur on the animal prevents heavy contamination of the skin. Thus, the practice of pre-operative shaving and skin preparation was given up and the skin shaved on the operation table just before operation. As a further precaution, penicillin has been used as a routine post-operatively since the four cases of wound infection occurred. As a result, no further cases of wound infection have taken place and 38 animals have been successfully operated upon.

The cases have been divided into the following groups for purposes of this study:—

(*a*) Five castrated male and five female animals, a total of ten animals, have been sacrificed four months after successful grafting operation. This series of cases were observed by taking *x*-ray photographs of the grafted areas of the femur and tibia at four weekly intervals. The changes which the grafts underwent were studied. It was found that in almost every case so followed up, there was *x*-ray evidence of complete absorption of the graft by the end of the fourth month from the time of the operation. These ten animals were sacrificed four months after the operation because it was felt that the best time to study the effects of the insertion of the grafts was when they were just completely absorbed or assimilated. Any further changes which may occur after this period, could not be logically attributed to the presence of the graft in the region of the metaphysis.

(*b*) Five castrated male and five female animals have been kept under observation for a much longer period. In the case of these animals, the intention has been to keep them under observation till all growth of bones ceases. Six of this group of animals reached the stage when the *x*-rays showed beginning fusion of the epiphysis with the diaphysis. This radiological epiphyseo-diaphyseal fusion can be taken as objective evidence of the cessation of all growth, and as soon as this begins to take place, for purposes of these experiments the animals may be considered to have ceased to grow. Four animals of this group still show full presence of the epiphyseal growth plates in their *x*-rays and so, they are still being kept under observation. They will be sacrificed as soon as radiological evidence of fusion appears.

(*c*) A group of six uncastrated male animals have been observed. Three animals of this group have been sacrificed four months after successful grafting operation. The other three animals still remain under observation.

(*d*) A group of eight animals have been sacrificed from the point of view of studying the local tissue changes which accompany the grafting procedure. Two animals each have been sacrificed at the end of the first, second, third and fourth months following successful grafting. The exposure was done under anæsthesia and the animals killed at the end of the operation so that the bones could be removed for further study. The whole lengths of the femur and tibia were carefully exposed, special attention being paid to the operated ends of the bones. The change in the soft tissues and the periosteum were studied by the naked eye. The normal limb was also similarly exposed and the local appearances of the two sides compared. The periosteum was then separated and the surfaces of the bone of both sides compared. The bones were then divided longitudinally to study and compare the appearances of the inside of the bones as well as the growth plates. Due to absence of facilities for colour photography, these changes could not be photographed. Careful photography would have given a very vivid picture of the differences observed between the two sides. Histological sections of the local tissues would also have been very valuable but there were no facilities for doing so. Hence, mere naked eye observations were made. This aspect of the work was not realized when the initial proposal was submitted nor when last year's report was made. It is now felt that these services are essential if the full potentialities of this work are to be exploited.

(*e*) A group of four animals have been operated upon by the same technique as the grafting method. In these animals only channels were prepared in the region of the metaphysis of the femur and tibia but no grafts were placed inside these channels. Two of these animals were sacrificed four months after operation and two still remain under observation as they are still growing. They will be sacrificed when growth in the bones ceases.

RESULTS OBTAINED.

1. First group of ten animals—five castrated male and five female, sacrificed four months from the date of the operation. This includes the four animals reported upon last year.

Table I shows the measurements of the femur and tibia of each animal. The figures for the normal as well as for the operated limb are shown side by side. Both the length as well as the diameters of the bones have been measured. It will be noticed from the chart that there was a definite increase in the length of the operated bones in each case, the total difference in length between the femur and the tibia amounting to an average figure of about 1 cm. It will also be noticed that the amount of lengthening in the femur is more than that of the tibia although contrary to human skeleton the femur is not much longer in length than the tibia in these animals. As a matter of fact, the final length of the tibia in goats often exceeds that of the femur. Perhaps this difference in the behaviour of the two bones is related to the difference in the amount of muscles that clothe and surround these bones; the femur being covered by fairly large-sized muscles while the tibia being relatively subcutaneous. This difference may influence the local tissue reaction caused by the insertion of the grafts.

The increase in the diameter of the operated ends of the bones has varied between 2 to 5 mm. only. Although these differences in length and diameter are small, they are significant as they have been observed in each of the animals operated upon. There is, however, one source of error which has not been taken into consideration so far but will be rectified at a later date. This applies to the possibility of the occurrence of spontaneous differences in the length and diameter of bones of the two sides during the period of growth. For this purpose, unoperated animals of different age-groups corresponding to the ages at which the operated animals have been sacrificed, should also be sacrificed and their bones measured carefully in order to find out if any difference in their lengths exist. This observation will be performed at a later date and the point clarified.

Table I.

Showing details of findings at post mortum—four months after grafting.

Period of observation, weeks	Length of operated femur, cm.	Length of normal femur, cm.	Difference in length, cm.	Length of operated tibia, cm.	Length of normal tibia, cm.	Difference in length, cm.	Total increase of growth, cm.
18	13·4	12·6	0·8	14·6	14·2	0·4	1·2
18	14·3	13·8	0·5	16·5	15·9	0·6	1·1
17½	13·8	12·9	0·9	14·9	14·4	0·5	1·4
17	14·0	12·4	0·6	15·2	14·6	0·6	1·2
16	12·4	11·8	0·6	13·8	13·4	0·4	1·0
17	12·6	12·2	0·4	13·9	13·4	0·5	0·9
16½	13·8	13·1	0·7	14·6	14·1	0·5	1·2
17	11·9	10·5	0·4	12·6	12·3	0·3	0·7
18	13·2	12·6	0·6	13·9	13·4	0·5	1·1
17½	12·8	12·1	0·7	14·0	13·7	0·3	1·0

In none of these animals the epiphyseal growth plates showed any radiological or macroscopic evidence of damage. There were no growth deformities in the grafted limbs. The articular end adjacent to the grafted side showed evidence of enlargement.

2. Second group of ten animals, six of whom have been sacrificed at the end of growth period:—

These six animals operated on in June-July last year, were sacrificed in June-July this year. All these animals showed evidence of epiphysio diaphyseal fusion starting in their bones. It was not felt necessary to wait for complete fusion of the epiphysis with the diaphysis as these animals do not keep growing for very long after a period of one year and whatever growth takes place is very nominal in extent. As most of the major portion of growth was already completed, it was felt that for purposes of this series of experiments, it was all right to sacrifice these animals at the end of twelve months.

Table II gives the measurements of the length and diameter of the femur and tibia of the two sides in these six animals. It will be noticed at once that in these animals there is no material difference in the length of bones of the two sides and even when a difference exists, the dispartiy is so slight that no importance can be attached to it. A difference in the diameter of the operated ends of the bones, still persists and the extent of difference is about the same as in the group of animals sacrificed at an earlier date. This means that acceleration of growth caused by the insertion of the grafts have not persisted. Once the grafts got absorbed and once normal growth conditions are established, further growth in the limbs has a tendency to equalize length of the bones.

TABLE II.

Period of observation	Length of operated femur, cm.	Length of normal femur, cm.	Difference in length, cm.	Length of operated tibia, cm.	Length of normal tibia, cm.	Difference in length, cm.	Total difference, cm.
12 months	17·2	17·2	*Nil*	21·0	21·0	*Nil*	*Nil*
11½ months	17·6	17·6	*Nil*	21·6	21·6	*Nil*	*Nil*
12 months	16·8	16·9	0·1	20·8	20·8	*Nil*	0·1
11½ months	17·8	17·8	*Nil*	21·8	21·8	*Nil*	*Nil*
12 months to 1 week	17·3	17·4	0·1	21·5	21·5	*Nil*	0·1
12 months	17·2	17·2	*Nil*	21·1	21·2	0·1	0·1

3. Observations in this group do not differ in any way from that of group (1). It is not possible to attach any importance to the total length of the bones in these cases. Although the animals were carefully selected from good stock, their pedigree is not guaranteed and it is not possible to dogmatize on the expected size they were to reach.

4. Eight animals sacrificed at different intervals from the date of operation and two sacrificed one month after operation. At this time there was a fairly well-marked reaction in the local tissues. The operated end of the bones felt thicker than the normal side and the areas felt somewhat warmer than the adjacent portion of the limb. The thickening was more noticeable at the lower end of the femur than at the upper end of the tibia. On dissection of the local area, many new vessels were encountered in the grafted area and these vessels bled more freely than the vessels of the opposite normal limb. The periosteum was thickened and there was vascular granulation tissue surrounding the area of the graft. The graft showed signs of partial absorption.

Exposure of the site of grafting eight weeks after the operation showed that the vascular reaction had subsided to a great extent. The clinical thickening was less than before and there was no difference in the temperature between the grafted ends and the surrounding areas of the skin. The graft showed further absorption and the drill holes made in the bone showed evidence of being filled up with granulation tissue. In this granulation tissue osteoid and later osseus tissue is laid down as shown in the serial *x*-rays taken at monthly intervals.

Exposure 12 weeks after the operation did not show any material remnants of the vascular reaction visible to the naked eye although *x*-rays taken at this stage still show that small remnants of the graft remain in the recipient area.

Sixteen weeks after the operation, exposure shows almost complete incorporation of the graft in the recipient area, filling up of the bone channels with newly laid bone, complete subsidence of the vascular reaction to naked eye examination and except for a slight thickening of the bone end, no evidence of the grafting operation remains. *X*-rays by this stage also show complete absorption or incorporation of the grafts. In one animal the tibial bone channel was not completely filled up at exposure.

5. Out of four animals in this series, two have been sacrificed. The measurements of the femur and tibia of the two sides are shown in the accompanying chart (*see* Table III). It will be noticed that mere operative trauma of a comparatively minor nature has no stimulating influence on the epiphyseal growth plates. Nor is such an operation likely to give rise to any marked or sustained vascular reaction.

TABLE III.

Duration of observation, weeks	Length of operated femur, cm.	Length of normal femur, cm.	Difference in length, cm.	Length of operated tibia, cm.	Length of normal tibia, cm.	Difference in length, cm.	Total difference, cm.
16	12·2	12·2	*Nil*	14·3	14·3	*Nil*	*Nil*
16½	13·0	13·0	*Nil*	14·6	14·6	*Nil*	*Nil*

DISCUSSION.

No simple, safe, satisfactory, reliable but effective method of stimulating the longitudinal growth of a long bone has yet been discovered. In this series of observations it is found that insertion of autogenous cortical bone grafts into channels prepared in the metaphyseal regions of the growing ends of long bones of young animals brings about a stimulation of the epiphyseal growth plates resulting in acceleration of growth. The increase in length obtained amounts to less than five per cent of the total length of the bone operated upon. This stimulation is not brought about by mere operative trauma to the metaphyseal region as animals who were not grafted but were otherwise submitted to the same operative procedure, failed to show any increase in length of the bones. The presence of the grafts had some specific effect in causing stimulation of growth. This may be the result of three factors:—

(*i*) The graft brings about a well-marked vascular reaction. This is related to nature's attempt in absorbing the graft. The vascular reaction in non-grafted experiments was nowhere as well-marked as in the grafted cases;

(*ii*) perhaps the graft supplies local osteogenic cells, and

(*iii*) the graft acts as a local depot for calcium salts required for accelerated growth from the epiphyseal growth plates.

All these factors produce a combined synergestic action on the growth plates causing it to multiply more rapidly than normally.

Besides stimulation of longitudinal growth, the grafting procedure also sitmulates circumferential growth of the operated ends of the bone. The increase in the circumference of the lower end of the femur and the upper end of the tibia, as compared to the opposite side, varies between 2 to 5 mm. only. This is not a large difference but the disparity has been noticed in each of the operated cases.

These findings showing evidence of stimulation of the activities of the epiphyseal growth plates were noticed when the animals were sacrificed four months after the operation, i.e., as soon as the grafts showed evidence of complete absorption.

In the control group, where the animals were observed for a much longer period and were sacrificed only towards the end of the growing age, i.e., at a time when the epiphysis and diaphysis were just beginning to fuse together showing evidence of cessation of growth in the limbs, the bones of the two sides no longer showed any disparity between their lengths. During the intervening period, in which growth occurred, while the grafts were already completely absorbed, the basic pattern set by the inherent growth potentialities of the limb bud and its osseous elements, asserted itself and equalization in the length of the limbs took place. The disparity which was caused by artificial stimulation of the growth plates was set aside.

Growth in limb bones is determined by hereditary, congenital, physiologic, endocrine, metabolic, nutritional and other factors but appears to be pre-determined in each individual unless it is altered by a pathological process and normal activity and normal blood supply helps to maintain this basic pattern. This basic pattern is so well fixed in the very cartilagenous nucleus of the limb that Fell in his classic experiments found that even if the limb bone is grown in an artificial medium, it has a strong tendency to grow to its set pattern. Local influences have a very slight effect in disturbing this basic pattern of growth. Where stimulation of growth occurs under abnormal clinical conditions, the pathological processes work for many years continuously to bring about the change in the limb growth.

Here, in this set of experiments, the local disturbances caused by the grafting process were not sustained in its effect and subsided within a very short time after the operation. Within eight to ten weeks of the operation, the increased vascularity of area of the operation subsided to a large extent. After 16 weeks of the operation, the vascularity of the local tissues had returned to normal. The graft, which supplied the specific stimulus to growth was also rapidly absorbed during this period. Besides that, as growth took place from the epiphyseal region, the graft was pushed further away from the vicinity of the epiphyseal growth plates and thus its influence as a stimulating agent abated. Thus, all the factors which act as stimulants to the epiphyseal cartilage rapidly lose their effect following the operation. The influence which they have on the cartilage is not sustained for enough length of time. The clinical cases on the basis of which these experiments were originally designed were cases where hard, adult cortical grafts were used. These grafts resisted penetration by vascular channels and, therefore, remained relatively intact inside the tissues for a very prolonged period. The autogenous grafts taken from young growing animals consist of rather thin, soft and non-resistant cortical bones. These grafts have a comparatively open structure. They are easily penetrated by the new vascular channels which grow in response to their insertion in the metaphyseal regions. Penetration is quickly followed by absorption and incorporation in the recipient area. As a result, the local tissue reaction also subsides rapidly.

Whereas, the bones become equalized in length following an initial increase, and the increase in length was not maintained as further growth in the limb took place, the increase which initially occurred in the circumference of the bone was maintained till the end of the period of growth. This increase was caused by deposition of subperiosteal bone from the deep osteogenetic layer of the periosteum. The diameter of the bone is regulated by the modelling effects of the metaphyseal region. In this case the increase occurs away from the actual point where the modelling process goes on. As such, the initial increase in diameter has persisted and has not been altered by further growth.

In none of the cases so far observed, whether at the end of the four-month period or towards the end of the period of growth, has there been any evidence of damage to the epiphyseal growth plates. The limbs on the operated side have continued to grow normally and have attained the same size and are functionally as perfect as the unoperated side.

It can, therefore, be concluded that under the conditions set up in this series of experiments, stimulation of the epiphyseal growth plates leading to acceleration in the growth of the bone takes place. Mere temporary acceleration of growth, however, does not ensure that the ultimate length of the limb will persist to be greater on the operated side. It is, however, reasonable to expect that if similar local tissue changes can be sustained for sufficient length of time and preferably throughout the period of growth, then actual lengthening of the limb is very likely to occur. The method here used is completely undamaging to the cartilagenous growth plates.

There is another aspect to be considered. We have found that in three to four months, while the graft remains effective as a growth stimulus, the total length of the limb increased by less than five per cent. If a five per cent increase in length is all that can be attained by this method, then the method would not be of great value for clinical purposes where larger extent of disparity is commonly met with requiring equalization. If, however, it is assumed that prolonged effect of similar local condition will keep up a similar rate of increase for a longer period then we may achieve anywhere up to 10 to 15 per cent of the total length of the limb. In such cases, the increase will be very valuable in clinical use. Total length of the limb here is computed as the combined length of the femur and the tibia. It is, therefore, worthwhile continuing with this type of experiments with a method which will have a more sustained local vascular response as in that case clinically useful degrees of lengthening may be achieved.

In the experiments to be performed in future, hard, cortical, homogeneous, preserved adult grafts will be placed in the metaphysis. Such grafts cannot be absorbed so rapidly. Also there is no strict limit to the amount of bone that can be inserted into the recipient area. Using autogenous grafts the quantity of bone to be used for grafting purposes is rigidly limited as very small quantities of bone can be taken out of the donor area from these young animals. The great quality of this series of experiments is that the method is completely safe. Most methods used in the past have proved to be damaging to the growth plates or have set up too violent a reaction in the tissues causing their extrusion. No such difficulties have arisen with this method. As such, it is possible that with some modifications in the details of experiment, the initial objectives set out in these experiments may yet be achieved.

15. Scheme for Tuberculin re-testing of B.C.G. mass campaign vaccinated persons in India under Dr. P. V. Benjamin, Adviser in Tuberculosis, Directorate General of Health Services, New Delhi.

In order to collect data on post vaccination tuberculin sensitivity and to determine how efficiently the groups are selected for vaccination according to the accepted standards for tuberculin testing and reading and to appraise the allergy produced by vaccinations done during mass vaccination campaign, WHO/TRO BCG Assessment Team conducted studies in India in the year 1954-55. The material collected by them is being statistically analysed in Tuberculosis Research Office, Copenhagen, and the final reports have not yet been received. A preliminay report, however, was published in WHO Bulletin, Vol. 12—No. 1-2. The main findings of this report are that:—

1. The pattern of pre-vaccination sensitivity differs in different parts of India.

2. Specific sensitivity as indicated by strong reactions to 5 TU tuberculin is found in all areas.

3. Low grades sensitivity evidenced by weak reaction to 5 TU and strong reaction to 100 TU which is considered non-specific was found more in the low lying humid areas than at higher altitudes.

4. The post vaccination allergy was found to be weaker than what was anticipated and it was also found to vary in different areas.

Subsequent investigations by the same team on the possible explanations for such a result show this weak allergy may not be due to the incapacity of the Indian children to produce strong allergy or weakness of Indian vaccine or impairment of vaccine due to exposure to light. However, it was felt that waning of allergy with time and personal variations in technique of vaccination may be contributed factors for this weak allergy.

To continue these studies an assessment team consisting of a doctor and a statistical clerk was recruited by the Indian Council of Medical Research and the work was started in August, 1955. In this assessment areas not covered by WHO/TRO team will be taken up first.

MATERIAL AND PROCEDURE FOR THE PRESENT STUDY.

Selection of groups.—Selection of groups for re-testing are made from previously vaccinated groups of school children for whom individual records are available. The sampling is done by the Statistical Officer of the Central BCG Organization. The following points are kept in view in sampling:—

1. That selected groups cover the widest possible geographical distribution in the country including groups from urban, semi-urban and rural areas.

2. That groups vaccinated with different batches of BCG vaccine are included, certain batch numbers being repeated.

Unvaccinated children are also included in the groups retested so that in reading the reactions the staff would not know which children had been vaccinated and which not.

Besides this, some unvaccinated groups have been tested and vaccinated by the team. This will enable us to compare at a later date the post vaccination allergy produced in these groups with that produced in groups vaccinated by mass campaign teams with the same batch of vaccine.

PROCEDURES.

Study of post vaccination allergy includes measuring of scars or lesions at the site of vaccination in previously vaccinated children and giving tuberculin tests (Mantoux) intracutaneous injection 0·1 c.c. of 5 TU of PPD supplied by the BCG Vaccine Laboratory, Guindy, in mid dorsal surface of left forearm.

Indurations are measured in millemeter after 72 to 96 hours; type of induration (according to hardness) and complications if any are recorded. The individuals with 7 mm. indurations or less are given intracutaneous injection of a higher close of tuberculin (0·1 c.c. of 100 TU) on the mid dorsal surface of the right forearm. Measurement of indurations in mm. type of indurations and complication are again recorded after 72 to 96 hours.

During the period under report the Research Officer, with the help of a clerk, carried out the work in the States of Kashmir, Himachal Pradesh, Saurashtra, Madhya Bharat and Bihar. With filling in of the posts of the BCG Technician and Statistical Assistant larger out-turn of work can be expected. The groups retested so far are school children of 5-18 years age, vaccinated with 13 different lots of vaccine in fifteen groups comprising of 43 schools. The particulars are shown in Table I.

As only two unvaccinated groups have been tested and vaccinated by the team, no remarks about the prevaccination allergy are considered appropriate. The present paper, therefore, deals only with the studies on Post-Vaccination allergy.

OBSERVATION

The mean indurations have been worked out for thirtyfour groups out of which four are combined groups comprising of 15 small schools. The results are shown in columns 9 and 10 of Table I. The overall mean induration for all these groups is 10·7 mm.

From the distribution of the mean post-vaccination indurations for different groups shown in Table II it would be seen that about 80 per cent of the mean indurations are in the range of 10 to 13 mm.

Columns 3 and 4 of the Table show the distributions of mean indurations separately for the 'old groups' and the 'recent groups'. In both the groups about 80 per cent of the mean indurations lie in the range of 10 to 13 mm. The remaining mean indurations are higher in case of 'old groups' and lower in the case of 'recent groups'. As there is no reason to believe that allergy produced by BCG vaccination becomes stronger with time natural infection after BCG vaccination may be one of the possible factors; but further investigations on this point is necessary.

TABLE I.

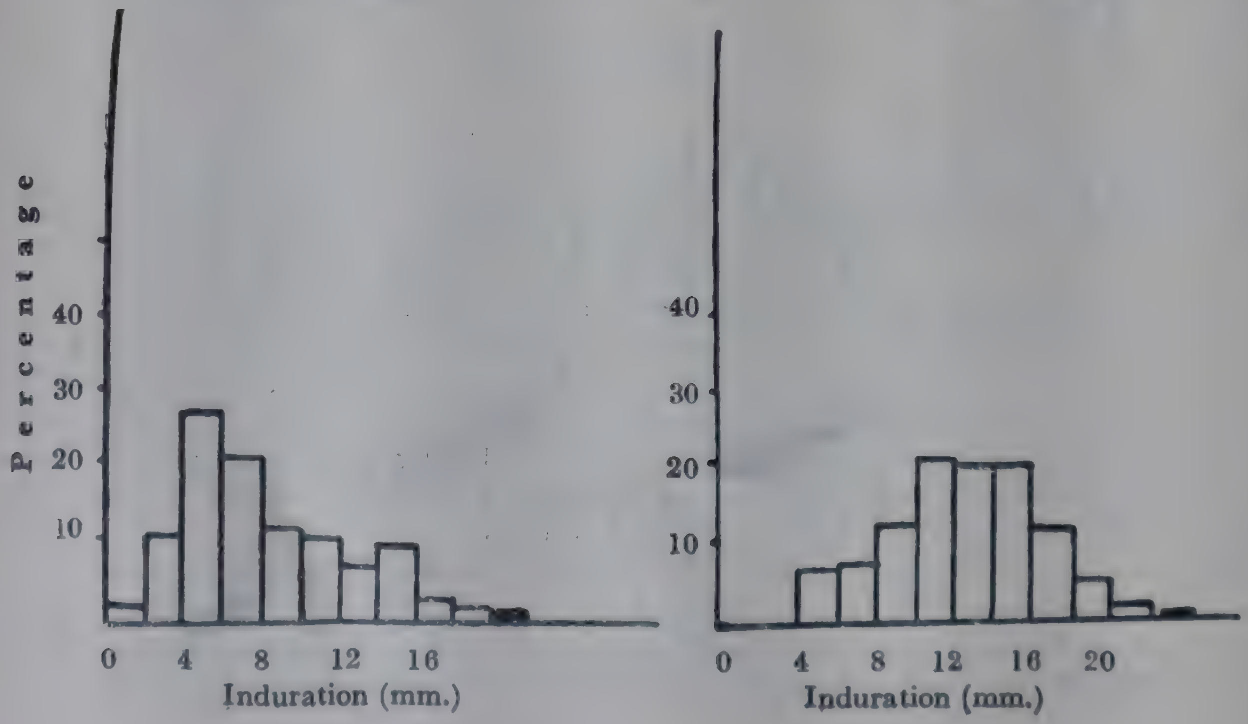
Serial No.	State.	Station.	Number of schools.	Vaccine batch number.	Number of months retested after.	Number of cards for which ditributions are made.		Mean indurations in mm.	
						Entire group.	Individual groups.	Entire group.	Individual groups.
1	2	3	4	5	6	7	8	9	10
1.	Jammu & Kashmir	Srinagar	1	421	14	81	...	9·1	...
2.	—do—	Srinagar	1	429	12	192	...	12·8	...
3.	—do—	(a) Barzala (b) Nagam (c) Charari-sharif	3	439	9	160	54 51 55	11·9	11·8 12·5 11·6
4.	Himachal Pradesh	Jogindernagar	2	389	22	243	177, 66	10·8	10·8, 10·9
5.	—do—	Sundernagar	3	340	32	192	123, 47, 22,	13·2	13·6, 11·8, 13·8
6.	Saurashtra	Junagadh	5	390	23	194	52, 70, 18, 35, 19	12·4	13·0, 12·4, 15·4 11·0, 11·0
7.	—do—	Porbandar	2	398	21	223	164, 59	10·1	10·4, 9·5
8.	—do—	Gondal	2 (four shifts)	423	15	185	69, 28, 23, 65	9·2	9·7, 9·1, 10·0 8·4
9.	—do—	(a) Dhrol (b) Jodiya	4	440	11	292	120, 35, 71, 66	9·7	9·0, 9·6, 9·6 11·0
10.	—do—	Botad	2	448	10	152	106, 46	8·3	9·6, 5·3
11.	Madhya Bharat	Indore	5	390	23	71		15·3	Not worked-Small No.
12.	—do—	Gwalior	5	378	26	128		11·2	...
13.	Bihar	Dhanbad	3	378	27	219	60, 69, 90	12·2	11·5, 12·8, 12·3
14.	—do—	Purulia	2	463	7	75		12·4	Not worked-Small No.
15.	—do—	Hazaribagh	3	486	2	125		11·9	...
			43			2532		10·7	

TABLE II.

Mean indurations in mm.	All groups.	" Old groups " (21 months or over).	" Recent groups" (2 to 21 months).
(1)	(2)	(3)	(4)
Less than 8	1	...	1
9	2	...	2
10	8	1	7
11	6	5	1
12	6	3	3
13	7	4	3
14	2	2	...
15	...	...	...
16	2	2	...
Total ...	34	17	17

A study of the distribution of post-vaccination indurations shows that the pattern of allergy apparently varies for different areas and batches of vaccine. Two clearly discernible types of distributions which have been noted are shown in Fig. I(A and B). It will be seen that there is larger percentage of smaller induartions (i.e. less than 7 mm.) in distribution IA as compared to IB. Such varying patterns of distributions were also observed in the WHO/TRO study. However, the variations noted in the present study are not as divergent as that in WHO/TRO study.

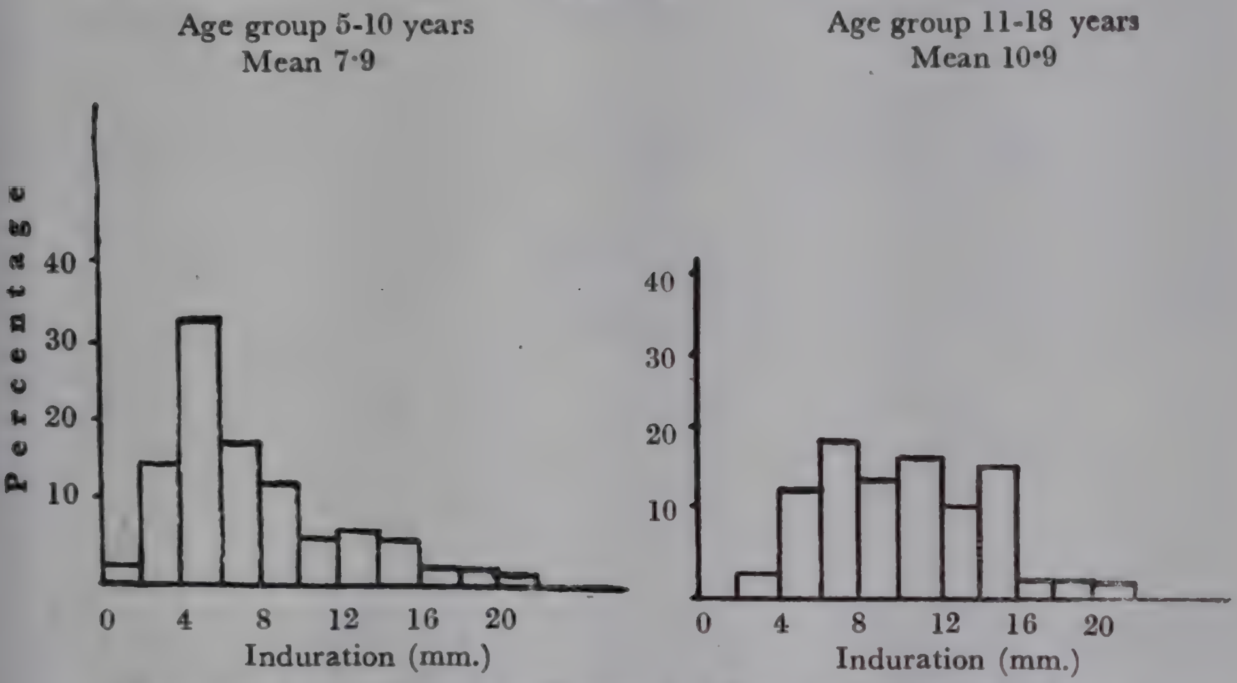
FIG. I


A. Jammu & Kashmir. Batch 429.
No. 192. Mean 8·5.

B. Himachal Pradesh. Batch 340.
No. 192. Mean 13·2.

With a view to finding whether the age group of the vaccinated children has any effect on the pattern of distribution, the data was further analysed according to two age groups, 5 to 10 and 11 to 18. This analysis has indicated that the pattern of distribution is likely to vary for the two age groups considered, the younger age-group showing a distribution with larger percentage of smaller reactions (i.e. similar to Figure IA). (The results for batch No. 429 are shown in Figure II). This difference in pattern between age groups, however, seems to be smaller for the groups vaccinated with the earlier batches of vaccine. The reasons for these variations are not evident, but natural infections after BCG vaccination may again be considered as a factor.

FIG. II


Age group 5-10 years
Mean 7·9

Age group 11-18 years
Mean 10·9

The groups considered in Figure I differ also in period after vaccination. As stated earlier this may be one of the factors responsible for the difference in pattern between Figure IA and IB.

The allergy produced by BCG vaccination in India, as indicated by the present study is neither so weak nor so divergent as that observed in WHO/TRO preliminary report. The latter report does not, however, indicate whether the distributions of the post-vaccination indurations have been analysed according to age groups and period after vaccination. It may be worth while to investigate how far these two factors can account for the variations noted in the WHO/TRO study.

That personal variations in the vaccination technique may affect the allergy produced has been observed in this study also. Two schools in one town were vaccinated with vaccine batch No. 448 on the same day by two technicians. On retesting the mean indurations for these two groups were found to be 9·6 mm. and 5·3 mm.

With regard to the waning of allergy with time, follow up investigations would be necessary. The material collected so far does not give any clear indication of the correlation between size of scar at the site of vaccination and post-vaccination allergy and of the relationship between altitude and tuberculin reactions to 100 T.U.

As the present study is based on rather limited data further investigations on the subject are necessary to confirm the above observations.

16. Studies on anti-fatty liver fractions of the pancreas and their róle in the prevention and treatment of fatty livers, under Dr. J. C. Sachdev at the M. G. M. Medical College, Indore.

Preventive action of the antifatty liver factor of the pancreas and lipocaic on ethionine induced (given intraperitoneally) fatty liver was studied in 25 normal female fasted albino rats weighing between 100 and 150 grammes which were divided into five groups, as under:—

Group number	Number of animals	State fasting	Compounds injected	Quantity per injection	Route of administration
I	5♀	Fasting	Ethionine	50 mg.	Intraperitoneal
II	5♀	,,	Ethionine *plus* Methionine	50 ,, *plus* 50 ,,	,, ,,
III	5♀	,,	Saline	2 c.c.	,,
IV	5♀	,,	Ethionine *plus* Lipocaic	50 mg. 50 mg.	,, ,,
V	5♀	,,	Ethionine *plus* A.F.L.F.	50 mg. *plus* 50 mg.	,, ,,

Dl Ethionine was injected intraperitoneally in five doses of 50 mg. each at interval of 2·5 hours, in all the groups except III. The supplement of antifatty liver factor and lipocaic in a similar dose as ethionine was given to group IV and V, respectively. Control group II received methionine as supplement in the same dosage. In the negative control group III animals were only injected with saline.

Normal animals receiving ethionine intraperitoneally in group I showed a marked increase of total liver lipids. An average value of 13·98 per cent wet weight liver was observed. Animals in group II receiving methionine and ethionine intraperitoneally in equal doses an average value of 7·2 per cent for total liver lipids was observed. Group III—control group—of animals receiving only saline by intraperitoneal route—an average total liver lipids value of 6·77 per cent was observed in this group. A marked increase in total liver lipids of group IV—animals receiving ethionine and lipocaic intraperitoneally in equal doses was observed. Average total lipids value of 13·69 per cent was obtained. ethionine *plus* A.F.L.F. intraperitoneally in group V also showed an increase in the liver lipids. Total liver lipids in this group gave an average figure of 13·25 per cent.

The analysis of the results in the above groups demonstrates that pronounced accumulation of fat can occur in albino rats by the administration of ethionine by intraperitoneal route and that antifatty liver factor and lipocaic fail to prevent this accumulation of liver fat when administered simultaneously with ethionine. Methionine, however, on the other hand, can prevent this accumulation under similar conditions. The average values of groups I, V and IV were higher by 92 per cent than those in groups II and III.

17. Experimental study of the effects of increase in portal vein pressure with reference to the development of ascites and its relation with blood flow in the hepatic artery under Dr. R. M. L. Mehrotra at the K. G. Medical College, Lucknow.

The pathogenesis of ascites in chronic liver disease has been the sabject of intensive study recently. A number of factors have been advocated to influence the collection of fluid in the peritoneal cavity, viz., increase of portal vein pressure, reduction in colloid osmotic pressure of blood, retention of sodium ions, and lymph stasis. The relative rôle of any single of these factors has, however, been much disputed. Increase of portal vein pressure, though considered by many as playing a major rôle, is denied by others. Rousselot (1940), Pattison (1949) and Blackmore (1947) report markedly elevated portal pressures in congestive splenomegaly without ascites. Their findings reveal that chronic portal hypertension may exist without there being ascites. The experimental investigations of Volwiler, Bollman and Grindlay (1950), on the other hand, suggest that ascites may occur in the absence of an elevated portal vein pressure. These workers believe that accumulation of fluid in the peritoneal cavity in chronic liver disease is due to the free pouring of lymph into the peritoneal cavity. The site of this fluid overflow has not been demonstrated. Ivers on (1950) considers the level of the colloid osmotic pressure of the blood as the major factor determining accumulation of fluid in the peritoneal cavity. Butt, Snell and Keys (1939), on the other hand, state that in most cases of ascites the decrease in colloid osmotic pressure is not conspicuous and that the lowered osmotic pressure is not the sole cause for the development of ascites. Changes in the permeability of peritoneal capillaries might be concerned in the production of ascites for serum albumin injected into the blood quickly passes out in the ascitis fluid (Kunkel, 1950).

Kunkel *et al.* (1948) and Kunkel (1950) consider an overall retention of sodium by the kidney as the cause of ascites. This retention of sodium is perhaps influenced by changes in the blood volume or as suggested by Kunkel (1950) by alterations in the adrenal cortical activity. Ralli *et al.* (1945) suggest that impaired inactivation of the antidiuretic hormone of the pituitary by the diseased liver is responsible for the retention of sodium.

The mechanism of the production of increase in the portal vein pressure in cirrhosis is frequently explained as due to mechanical obstruction to the flow of blood in the scarred liver. Recent experience from studies on liver biopsies in cases of portal hypertension suggests that there is no relationship whatsoever, between the degree of portal hypertension and the severity of liver scarring (McMicheal, 1950). Factors, such as anatomical abnormalities at the porta hepatis, distention of vascular arrangements inside liver also influence the pressure in the portal vein. It is possible that as a result of abnormal communications between hepatic arterial branches and portal tributaries, the normal mechanism which equalizes the arterial and portal vein pressures at the intra-hepatic junctions of these two blood streams is upset and the load of the arterial pressure embarrasses the venous inflow. It is on such hypothesis that ligation of hepatic artery has been advocated as a procedure to reduce the intensity of portal hypertension in chronic cases.

In the present study an attempt has been made to study rôle of portal hypertension on the development of ascites produced in rats after using various experimental procedures.

EXPERIMENTAL PROCEDURES.

In all 77 healthy young albino rats of both sexes have been used in this study. The animals were fed on a synthetic diet and given water *ad lib*. The operative procedures were carried out under ether anæsthesia and aseptic conditions. The peritoneal cavity was opened by a midline abdominal incision and following procedures adopted.

I. *Partial ligation of inferior vena cava above the hepatic veins:*

In 41 rats the liver was gently pulled and the portion of the vena cava between the liver and diaphragm carefully exposed. The vena cava was ligated tightly over an iron wire having a uniform diameter of 1·25 mm. and after the ligature had been applied the wire was gently removed. This procedure reduced the lumen of the vena cava to about 1/3rd of its original diameter. The peritoneal cavity was then swabbed, abdomen closed in two layers and the animals returned to their cages.

The mortality in this group was high; ten rats died within a few hours of operation, while another group of ten rats remained drowsy and died at irregular intervals varying from 24 to 48 hours. The remaining 21 animals were sacrificed at 1, 2, 7 and 28 days.

II. *Partial ligation of inferior vena cava below the hepatic veins:*

This was done in six rats. The vena cava was ligated over a wire 1 mm. in diameter immediately below the entrance of the hepatic veins. The procedures were similar as in (I). One animal died at 24 hours, while the remaining five rats were killed two days after the operation.

III. *Partial ligation of portal vein:*

In 15 animals the main portal vein was ligated over a wire 0·75 mm. in diameter using the procedures described above. This reduced the diameter of the vein to about 1/3rd of its original. Five animals died within 24 hours and the ten rats which survived, were sacrificed two days after the operation.

IV. *Partial ligation of portal vein and inferior vena cava below the hepatic veins:*

In 15 animals the procedures described in Nos. (II) and (III) were combined. Four animals died within 24 hours, while the remaining eleven rats were sacrificed two days after the operation.

In all these experiments, portal vein pressures were recorded at the time of sacrificed the animals. The pressures were taken in a specially devised null-point water manometer. Blood was collected for estimation of plasma proteins and plasma sodium. The animals were carefully examined for the development of ascites and complete chemical and microscopic study of the ascitic fluid was made in cases where ascites were present. Histological examination of liver and spleen was done in all instances.

RESULTS.

Before starting these experiments data regarding plasma proteins and pressures in the portal vein and inferior vena cava in 22 normal healthy albino rats were collected. Our findings show that the total plasma protein in normal rats range, from 5·2 g, to 7·35 g. per cent with a mean of 6.33 g. per cent. The pressures in the portal vein and inferior vena cava vary from 7 to 13 cm. and 2 to 4 cm. of water, respectively.

I. *Partial ligation of inferior vena cava above the hepatic veins.*

The occurrence of ascites after partial ligation of inferior vena cava above the hepatic veins is shown in Table I:—

TABLE I.

Days after operation	Total number of animals	ASCITES:	
		Present	Absent
1 ...	5	3	2
2 ...	5	5	...
7 ...	5	4	1
28 ...	6	1	5
Total ...	21	13	8

At one day, three animals out of five had ascites. The volume of ascitic fluid ranged from 1 to c.c. 2 c,c. The fluid was grossly tinged with blood and its protein content varied from 3·3 g. to 4·2 g. per cent. The pressures in the inferior vena cava were raised in all the animals, while the portal vein pressures were raised only in two animals without ascites. The total plasma proteins were within normal limits in four animals, while in one of the animals showing ascites it was slightly decreased.

At twó days, all the five animals showed ascites, the volume of fluid varied from 1 c.c. to 5 c.c. and its protein content from 3·45 g. to 4·4 g. per cent. The fluid was mixed with blood. Pressures in the portal vein and inferior vena cava were raised in all the animals and the plasma proteins were within normal limits.

At seven days, four animals out of five had ascites. The volume of ascites fluid varied from 2 c.c. to 22 c.c. The fluid contained only a few red blood cells and its protein content ranged from 2·6 g. to 3·8 g. per cent. Pressure in the portal vein and inferior vena cava was raised in four animals, while in one animal with ascites pressure readings could not be taken as the animal died during anæsthesia. The total plasma proteins were decreased in animals with ascites.

At 28 days, only one animal out of six had ascites. The ascitic fluid was clear and measured 5 c.c. Its protein content was 4·8 g. per cent. The plasma proteins in this animal were normal. The other five animals which did not show any peritoneal effusion had normal plasma protein concentrations. Pressures in the portal vein and inferior vena cava were raised in all the six animals.

Macroscopic findings in liver.—At one day, the liver was intensely congested, its surface was smooth and moist and showed small infarcts. At two days these changes were more marked. By seventh day the superior surface of the liver was found adhered to the diaphragm and in some animals the organ also showed adhesions with the omentum. The capsule appeared thickened and the organ was slightly shrunken in size. At 28 days, the liver was small and shrunken with a markedly thickened capsule; its upper surface was intimately fused with the disphragm and the margins of the liver lobes were rounded. On cutting, the liver appeared firm and had a dry shrunken appearance.

Microscopic findings in liver.—At *one and two days*, the liver showed marked œdema of the Glisson's capsule, extravasations of blood in the subcapsular region and small infarcts. The latter were mostly situated beneath the capsule. The sinusoids were markedly dilated and many of them were seen opening below the œdematous capsule. The portal and central veins were dilated and the endothelial cells lining these vessels appeared prominent. The liver cells showed fatty change in the periportal region. This change was well pronounced at two days.

At seven days, the capsule of the liver appeared thickened with increase of collagenous fibrous tissue and showed infiltration by small number of chronic inflammatory cells. The hepatic and central veins were thickened due to proliferation of endothelial cells and increase in the collagen content of the media. The portal veins did not present any marked change. The infarcts in the parenchyma were surrounded and invaded by granulation tissue and macrophages. Small strands of collagen fibres were seen extending from the region of the central veins into the liver parencyma. The liver cells in some of the animals showed fatty change, while in others it was absent. There was hyperplasia of Kupffer cells in some instances.

At 28 days, the capsule of the organ was markedly thickened and collagenous. The parenchyma of the liver was traversed by thin strands of fibrous tissue radiating from the central veins. The liver cells appeared swollen with prominent cell membrane and markedly fenestrated granular cytoplasm. Their nuclei were round and vesicular. The intimal layers of the hepatic and portal veins were thickened. Small number of inflammatory cells were evident in the periportal region. At 28 days there was, however, no evidence of fatty change in the liver cells.

II. *Partial ligation of inferior vena cava below the hepatic veins:*

The five animals of this group sacrificed at two days did not develop ascites. In two of them, however, slight œdema of the limbs and scrotal region was observed. The pressure in the inferior vena cava was raised in all, the readings varied from six to 14 cm. of water. The pressure in the portal vein was normal and ranged from 8 to 11·5 cm. Plasma protein estimations revealed normal value. Microscopically the liver in these animals presented normal appearances.

III. *Partial ligation of the portal vein:*

Only one animal of this group showed a small amount of peritoneal effusion (2 c.c.) though the portal vein pressures were increased in all; the pressure readings varied from 17 to 36·5 cm. Pressures in the inferior vena cava were normal. Plasma proteins did not show any deviation from normal. At autopsy, the tributaries of the portal vein were dilated and engorged with blood.

Microscopic examination of liver showed mild fatty change in two animals, small areas of patchy necrosis in one and massive infarction of one of the liver lobes in another animal.

IV. *Partial ligation of portal vein and inferior vena cava below the hepatic veins:*

Of the 11 animals sacrificed at two days, small amount of peritoneal effusion was evident in two rats. The volume of effusion was 0·5 c.c. and 1·5 c.c., respectively. The fluid was slightly tinged with blood and its protein content was 1·65 g. and 1·6 g. per cent. Pressures in the portal vein and inferior vena cava were markedly raised in all the five animals. The readings of pressure in portal vein ranged from 23 to 36 cm. and that in inferior vena cava from 8 to 17 cm.

Plasma proteins were normal. At autopsy the liver appeared normal; spleen showed congestion and a hæmorrhagic infarct in one animal. Microscopic examination of liver showed normal appearances.

SUMMARY OF FINDINGS.

Our findings show that partial ligation of inferior vena cava above of the liver is followed by massive outpouring of protein rich fluid in the peritoneal cavity. Incomplete ligation of inferior vena cava below the liver is without any effect. When the portal vein and inferior vena cava are both ligated below the liver or when partial ligation of portal vein alone is done, some fluid does appear in a small number of animals but the effusion is never profuse. The findings suggest that for the experimental production of ascites procedures which result in venous stagnation in liver are more successful than those involving accumulation of blood in the protal or systemic veins. This study further demonstrates that pressures in the portal vein and inferior vena cava were markedly raised in a number of animals without there being any evidence of ascites. Obviously, a high portal vein pressure can not be the only cause for the development of ascites in these animals. Our results also show that the colloid osmotic pressure of plasma proteins does not appear to play any significant part in the causation of ascites in these animals.

18. Studies of the role of immunologic mechanisms in the production of aplastic anæmia under Dr. V. S. Mangalik at the King George's Medical College, Lucknow.

The object of this study is to induce hypoplastic changes in the bone marrow of experimental animals, involving the cells of erythroid, myeloid and thrombocytic series either singly or together. For this purpose antisera will be prepared against these elements of blood and administered. The mechanism of the production of the hypoplastic or the aplastic change in the bone marrow so induced would be investigated.

In the first phase of this inquiry, we have started with studies on the effects of anti-erythrocytic serum on the blood and bone marrow of guinea-pigs. Rabbits were immunized against the red cells of guinea-pigs of mixed strain. The blood was obtained from cardiac puncture in guinea-pigs in tubes containing sodium citrate as anticoagulant. Erythrocytes were washed six to eight times with normal saline, re-suspended in it and injected into the marginal ear vein of rabbits. The following schedules of immunization were employed:

Schedule of immunization of rabbits with 50 per cent suspension of guinea-pig erythrocytes.

Day of injection	Quantity injected	Route of injection
1	1·0 ml.	I.M.I.*
3	2·0 ml.	,,
5	3·0 ml.	,,
7	4·0 ml.	,,
11	5·0 ml.	,,

*Intramuscular injection.

Schedule of immunization of rabbits with ten per cent suspension of guinea-pig erythrocytes.

Day of injection	Quantity injected	Route of injection
1	5·0 ml.	I.V.I.
6	8·0 ml.	I.P.I.
11	12·0 ml.	,,
16	15·0 ml.	,,
21	20·0 ml.	,,

I.V.I. = Intravenous injection.
I.P.I. = Intraperitoneal injection.

The animals were bled five days after the administration of the last injection. The titre of the hæmolytic serum was determined according to the technique described by Kabat and Mayer (1948).

The effects of the administration of immune serum on the blood of guinea-pig is under study. We have, however, observed that the degree of anæmia resulting after the injection of the serum varies in character according to the size and number of doses. The route of administration also determines the effect produced. Following a single large dose intravenously, the recipient animal dies within 24 hours of the administration of the serum. No evidence of hæmolysis is detectable in the peripheral blood. This effect appears to be ' toxic ' in nature. Repeated culture of the serum for the presence of any micro-organisms has been negative.

We are in the middle of our study of administration of repeated small doses of immune serum. There are indications that during the first days of administration volume changes take place in the red blood cells. Parallel with these is decrease in resistance to hypotonic solutions of sodium chloride. This period is followed by one of the destruction of red cells accompanied with intense reticulocytosis.

19. Study of incidence and relationship of prenatal and neonatal syphilis abortions, still-births, neonatal deaths and congenital syphilis and results of treatment with penicillin at various stages under Dr. B. B. Gokhale at the Sassoon Hospitals and B. J. Medical College, Poona.

Synopsis:—Five hundred and twenty-four women have been clinically examined at the antenatal clinic and their bloods have been submitted to STS (serological test for syphilis). Fifty-six cases with interesting histories and STS are being observed. Eighteen of these have been followed on to the maternity wards. Seven hundred and fifty-six mothers from maternity wards have been examined serologically and clinically along with their babies. Eighty-nine of them are being followed to their homes. Forty-five mothers, four husbands and three children with definite evidence of syphilitic infection have been treated with adequate doses of penicillin.

The number of patients antenatally examined, clinically and serologically at the Sassoon Hospital, has been five hundred and twenty-four. Out of these 56 are kept under closer observation because of interesting clinical and/or serological findings. Records of clinical examinations and histories of all these cases are maintained and are correlated with the serological tests. Serologically positive patients are treated along with their spouse whenever possible. The total number of expectant mothers so treated has been thirteen and the number of husbands has been one. These cases are followed on to the maternity wards and to assess the results of the treatment; cord or fontanelle blood of their babies is submitted to STS. The number of such deliveries has been eighteen.

All the non-registered or emergency cases are investigated clinically and serologically and the results of serological examinations of the babies' cord or fontanelle blood and mothers' veinous blood are correlated with their histories. The number of such cases has been 756. When positive serologically, attempt is made at serological examination of their husbands, and the results are correlated. Parents and babies with positive STS are given requisite anti-syphilitic treatment with penicillin and are followed to their homes. Eighty-nine such cases are at present under observation.

As many post-mortem examinations as are available are performed in cases of fœtal losses and notes of such post-mortem examinations are maintained. Six such post mortems have been done so far.

Products of conceptions in cases of abortions are examined histologically and these findings are correlated with the results of parauls' S.T.S. Number of such examinations during the period of study has been twenty-seven.

X-ray pictures of long bones of two interesting cases have been taken.

N. B. Each blood is submitted to at least two serological tests for syphilis (STS).

20. Inquiry into the rôle of tuberculosis in the pathogenesis of pelvic inflammatory lesions under Dr. P. K. Malkani at the Lady Hardinge Medical College, New Delhi.

A careful pelvic examination is made of all the patients attending the gynæcological out-patients department. Those suffering from inflammatory piseases are referred for study. They are divided in two major groups.

I. Cases of erosion of the cervix and chronic cervicitis.

II. Cases of adenexal inflammation.

Investigations are done on the following lines:—

A. *Histopathology of*

1. Biopsy of the endometrium.
2. Biopsy of the cervix in cases of erosion and endocervicitis.

B. *Bacteriological investigations for evidence of* Mycobacterium tuberculosis:

1. Ziehl-Neelson stain of discharge of the cervix.
2. Ziehl-Neelson stain of endometrial aspiration smear.

TECHNIQUE OF ENDOMETRIAL ASPIRATION SMEAR.

The patient is placed in lithotomy position. A vaginal speculum is inserted in the vagina. The cervix is exposed and swabbed with a mild antiseptic. A pohythelene tube with a syringe attached to one end is passed through the cervical canal into the uterine cavity. Uterine secretion is aspirated and then transferred to a slide. A smear of this secretion is made. This procedure can be repeated as often as desired without causing discomfort to the patient.

3. Ziehl-Neelson stain of the endometrial tissue and cervix tissue of the histologically positive cases of tuberculosis.

4. Culture of endometrial biopsy and cervix-biopsy of histologically positive cases of tuberculosis.

RESULTS OF STUDY DONE SO FAR.

	Total number	T.B. positive
1. Patients studied	53	13
2. Histopathology of endometrial biopsies including repeat examinations	86	21
3. Histopathology of cervix biopsy	8	1
4. Ziehl-Neelson stain of endometrial tissue ...	13	*Nil*
5. Ziehl-Neelson stain of cervix biopsy ...	1	*Nil*
6. Ziehl-Neelson stain of cervix discharge ...	76	*Nil*
7. Ziehl-Neelson stain of endometrial aspiration smear	57	*Nil*
8. Culture of endometrial biopsy for growth of *mycobacterium tuberculosis*	6	3
	(Report of 3 is awaited)	

Histological evidence of endometrial tuberculosis ...	13 cases
Histologial evidence of cervix tuberculosis ...	1 case
Culture of endometrial biopsy positive for growth of *Mycobacterium tuberculosis*	3 cases

Cases of adenexal inflammation are divided into following groups:—

	Number of cases	T.B. positive
1. Unilateral thickening	15	1
2. Bilateral thickening	2	*Nil*
3. Unilateral tubo-ovarian palpable mass ...	19	6
4. Bilateral tubo-ovarian palpable mass ...	13	6

21. Inquiry into the virulence to guinea-pigs and mice of tubercle bacilli isolated from tuberculous patients prior to, during and after treatment with streptomycin, PAS and isoniazid under Dr. Frimodt-Moller at the Union Mission Tuberculosis Sanatorium, Arogyawaram, P.O., Chittoor District.

Primary trials with liquid media, such as Kirschner, Sauton's and Besredka's media were done. As it was not possible to obtain a satisfactory growth in a short time, it was decided to use Lowenstein-Jensen medium as standard medium. For isolation of tubercle bacilli from specimens of sputa, stomach-wash and laryngeal swab cultures Lowenstein-Jensen medium was, therefore, employed.

The primary cultures were sub-cultured one, two or three times until good growth was obtained within two weeks or so. The culture was picked off and emulsified in small test-tubes with saline. After a good suspension had been obtained and coarse clumps had time to settle at the bottom, the supernatant fluid was withdrawn with a pipette and placed in special tubes which fit an electro-photo colorimeter. The density of the suspensions were compared with suspensions of barium sulphate which correspond to different dilutions of dry culture of tubercle bacilli. The suspensions were then diluted so that a series of dilutions with graded doses were obtained ranging from 10^{-3} down to 10^{-6} mgm. per c.c. For each virulence test two guinea-pigs were inoculated intraperitoneally with each dose mentioned, giving a total of eight animals for each virulence test. At the same time series of Lowenstein tubes were inoculated with the same suspensions in order to determine the number of viable bacilli per 10^{-6} mg. culture. The guinea-pigs were weighed every week. In the fourth week the guinea-pigs were tuberculin tested with 100 TU PPD. One guinea-pig of each pair was sacrificed at four weeks and the post-mortem findings noted. The remaining four guinea-pigs were re-tested in the eighth week and sacrificed after eight weeks.

At post mortem the amount of tuberculosis seen in the omentum, pyloric and hepatic lymph nodes, spleen, liver, sternal glands and lungs was noted. Parts of the organs were taken for microscopy and culture.

The extent of tuberculosis found was classified as follows:—

0 —No tuberculosis.

I —Tuberculous granulation tissue in the omentum.

II —Tuberculous granulation tissue in the omentum and involvement of the hepatic lymph node.

III —Tuberculous granulation tissue in the omentum and involvement of the hepatic lymph node and a few tubercles in the spleen.

IV —Moderate generalized tuberculosis, i.e., a few tubercles in the liver and/or lungs, besides of the omentum and the spleen.

V —Severe generalized tuberculosis.

Thirty-eight strains have been examined. The results are shown in the table.

The assessment of the virulence has been based upon the results found on all eight guinea-pigs, i.e., at four weeks as well as at eight weeks. A detailed report will be submitted later on. In some cases it has been necessary to repeat the virulence test, especially when the bacilli were found to be of very low virulence. Then a new series of guinea-pigs have been inoculated with 10^{-1} mg. and 10^{-2} mg. culture.

It will be noted from the Table that the virulence of strains from fresh untreated cases showed some variation as 5 out of 14 primary strains were definitely below standard virulence. The same was seen in one case out of nine sensitive strains from treated cases.

Of the 14 resistant strains 6 were avirulent and 5 attenuated.

Cultures from the inoculated guinea-pigs have been recovered and have been injected into another series of animals to see if the attenuation was stable or whether the strains could be induced to resume their previous virulence. All the strains recovered from the guinea-pigs have been tested again for drug sensitivity.

TABLE.

Results of virulence test.

Type of strain	Highly virulent	Virulent	Attenuated	Avirulent	Total
H37 RV	...	1	...	...	1
Primary culture from untreated case	2	7	5	...	14
Treated cases, strains sensitive to all drugs ...	1	7	...	1	9
Strains resistant to streptomycin	...	3	1	1	5
Strains resistant to INH ...	...	...	1	3	4
Strains resistant to streptomycin and INH ...	...	...	3	2	5
Totals	3	18	10	7	38

It will be quite obvious that this material must be increased several times before a full picture of the variety in virulence of strains from untreated as well as treated cases can be gathered. It would be of value to build up a series of investigations at two to three monthly intervals from the same patients.

As for the virulence towards mice, it has not yet been possible to get enough supply of mice to start the tests but 12 mice were secured this summer and they have been bred so much that it should soon be possible to start with mice also.

22. Inquiry on the elimination of acid-fast saprophytes from routine cultures for the *Mycobacterium tuberculosis* under the Director, Tuberculosis Centre, New Delhi.

As a preliminary thirty cultures of chromogenic acid-fast saprophytes grown on solid media were studied using dilutions of the cultures in distilled water. Mostly the organic acids were used to study the inhibiting effect on these saprophytes. Parallel control tests were put up with each chemical agent by substituting suspensions of H37 RV strain of the tubercle bacillus.

The initial study during the period under review has negatived the possible inhibiting action of some of the reagents studied at the concentrations used in the experiments. One of the reagents, viz., one per cent $KMnO_4$ in three per cent H_2SO_4, recently tested in the pilot experiment, has given promising results in that it has a greater inhibitory effect on the saprophytes than on H37. This will be studied in more detail.

Cultures of H37 RV and the chromogenic acid-fast saprophytes were grown on the Harefield modification of the Medlar and Sasano's medium. The growth on the surface of the medium was removed with a loop and transferred to a watch-glass kept in a Petri dish which was previously weighed. The weight of the culture was determined. This culture was then transferred to a mortar and ground for ten minutes with sufficient distilled water to give a suspension containing 1 mg. of bacilli per c.c. Further dilutions were made from this suspension to represent a tenth and hundredth of a mg./c.c. Sterile McCartney bottles were set up in racks, the number of bottles to correspond to the units of time periods of exposure to be tested, at the rate of one bottle for each time period. Into each of the bottles were placed 1 c.c. of a suspension of the saprophyte (1 mg./c.c.). A sterile solution of the reagent to be tested was then added in one c.c. amounts to each of the bottles, excepting the last bottle in each rack which received 1 c.c. of sterile distilled water to serve as a control. The time of exposure of the reagent was spaced and when the reagent had been allowed to act for the required period, further action of the reagent was stopped by the addition of a suitable neutralizing agent which was previously determined for each reagent and the total volume in each bottle was made up to 10 c.c. with sterile distilled water. From each bottle 0·1 c.c. amounts were transferred to two tubes of Medlar's media, so that each medium tube received 1/100 of a mg. of the bacilli. The media tubes were then incubated, the period of incubation in the case of saprophytes being four weeks at room temperature.

With each concentration of the reagents a control experiment outlined as above was performed substituting H37 RV suspensions for the saprophytes suspension, incubation in this case being carried out in the incubator and the period of incubation being six weeks.

Readings were recorded once in every three or four days in the case of saprophytes, and once a week for six weeks in the case of tubercle bacillus.

23. Inquiry on the incidence of human and bovine type of infection in extra pulmonary tuberculosis with particular reference to abdominal tuberculosis with or without associated pulmonary tuberculosis under Dr. N. L. Chitkara and Dr. M. L. Sur at the Medical College, Amritsar.

Fourteen cases of abdominal tuberculosis, which were being investigated already jointly under Dr. K. L. Wig, and Dr. N. L. Chitkara, were taken up. Thirty-nine new cases have been taken up since the commencement of this inquiry from 15th April, 1955, but six of these cases could not be investigated as they did not co-operate and left the hospital. So this report deals with 47 cases which have been classified as follows:—

Abdominal	31	
Abdominal with tubercular lymphadenitis ...	6	
Glandular	6	
Glandular	2	(these cases had abdominal symptomatology, but their barium study was negative).
Meningitis	1	
Polyserositis	1	

The following investigations have been done on these cases:—

1. Mantoux test was done on 45 cases. Forty-four were positive.

2. Radiological examination of chest was done in 43 cases, 26 cases showed no abnormality, five showed pulmonary disease, and the remaining cases either showed old healed pulmonary lesions or enlarged hilar glands.

3. Barium meal and enema skiagraphy of gastro-intestinal tract was done in 29 cases, 18 showed evidence of obstruction, 1 showed abnormality of mucosal pattern and the remaining ten showed no abnormality.

4. Gastric lavage specimens and sputa of all the cases were cultured, but no growth of *M. tuberculosis* was obtained in these cases.

5. Radiological screening of the chest of relatives of 34 cases was done. Only two showed pulmonary lesions.

6. Biopsy material from 39 cases was put up for culture. Eight gave positive cultures. Five of these neither killed the guinea-pig nor the rabbit. The results of remaining three are still awaited.

7. Histopathological examination of biopsy material was done in 29 cases. Twenty cases showed tuberculous lesions, while the remaining nine showed no evidence of tuberculosis.

8. Endometrial biopsy was done in 14 cases, but histopathological examination showed no evidence of tuberculosis.

9. Stool culture for *M. tuberculosis* were put up in 25 cases of abdominal tuberculosis, but no growth was obtained.

The negative culture and the avirulence of the *M. tuberculosis* to the guinea-pigs seems to be due to the use of the modern anti-tubercular drugs by the patients. This question will be discussed in the report of the next year, when we will have studied enough number of patients to draw conclusions.

Work done relating to 39 *new cases.*—The present series was started on 15th of April, 1955. In all 39 cases were taken up till 30th September, 1955; out of which seven cases were discarded as they could not be proceeded with. Later on one case, however, became available for further investigation. Thus the present summary refers to 33 cases in all.

Clinical diagnosis.—Out of a total of 33 cases, 17 were diagnosed clinically as tubercular abdomen alone, while in another group of six cases there was associated tubercular lymphadenitis. Thus in all abdomen was involved in 23 cases. Isolated tubercular lymphadenitis was diagnosed in eight cases. One case suffered from tubercular meningitis and one case had polyserositis.

Age incidence.—The ages of the patients at the start of the illness varied from seven years to 37 years and distributed as under:—

Below 10 years	4
Between 10 and 20 years	18
Between 20 and 30 years	10
Between 30 and 39 years	1

Duration of symptoms.—Before coming to the hospital the patients were having symptoms of disease for a varying length of time. The duration of symptoms varied from ten days to eight years, and are tabulated as follows:—

Under one month duration ...	1
From one to three months	11
From three to six months ...	1
From six months to one year ...	5
From one year to two years ...	3
From two years to five years ...	9
Above five years	3

Sex incidence.—Out of 33 cases, 26 were females and 7 males.

Various investigations done.—Gastric lavage.—An attempt was made to do the sputum examination but in the cases who had no sputum, gastric lavage was done. The specimens were examined by concentration and culture method for AFB. The total number of gastric lavage specimens examined was 55 in 24 cases. Attempt was made to examine three specimens in every case but in some cases this examination could not be done due to various reasons. All the specimens were negative for AFB.

Mantoux test.—One in 10,000 dilution of old tuberculin was used for the test. All cases excepting one gave positive result. In the negative case the test repeated with 1 in 1,000 O.T. was also negative. The histopathology of the mesenteric glands in this case showed no evidence of tuberculosis.

Radiological investigations: Lungs.—It was attempted to take the skiagram of chest in all the cases, but in those in whom it could not be done, screening of the chest was done. Radiological examination could not be done in two cases. Screening has been done in three cases and in the rest skiagram was done. Out of 31 cases thus examined no abnormality was detected in 18 cases. Amongst the rest, five showed evidence of mediastinal or bronchial glands enlargement, two healed calcified pulmonary primary complex, four infilteration or definite lesions in the lung fields, one thickened pleura, and one pleural effusion. One case who had no abnormality in the chest showed calcified nodes in the abdomen on a plain skiagram.

Barium meal study of G.I. tract.—It was done only in suitable cases of abdominal disease. In all it was done in 20 cases. In 17 cases it showed positive evidence of obstruction or filling defect. In one the result was doubtful and in two it showed no abnormality at all.

Barium enema study.—This examination was also carried out in suitable cases of abdominal disease only. In all it was done in 17 cases. In eight it showed evidence of obstruction, in one abnormality of mucosal pattern of cæcum, while in eight the test was negative.

Investigations of the relatives.—The relatives of the patients were subjected to screening of the chest. Only those cases could be examined who were either local inhabitants or could come from moffusils, for the investigations. Thus relatives of 20 cases have so far been examined and investigation showed positive results in two cases only.

In one case the sister of the patient showed enlargement of paratracheal glands with positive 1 in 10,000 Mantoux test. In the other case the mother of the patient showed lesions in both the lungs.

Histopathological examination.—Material for histopathological examination came from excision of tubercular gland from cases of lymphadenitis, or mesenteric glands at the time of performing lateral anastomosis in cases of iliocæcal disease; and from intestinal portion after hemicolectomy. Out of 18 specimens thus examined 14 showed tubercular pathology, while four cases showed no evidence of tuberculosis. Z. N. staining of sections failed to reveal the presence of AFB as such.

Culture for AFB.—Usually the material which was put up for histopathology was also mashed and pulped and put up for culture for AFB. In addition specimens, such as ascitic fluid, joint fluid, and CSF were also put up for culture. In all 25 specimens have been put up so far. Out of these, four are yet less than six weeks old.

Out of the 21 specimens which were put up for culture more than six weeks ago only three have given positive cultures. These are being further investigated by sub-cultures and animal inoculation. One guinea-pig and two rabbits are being used for each case for the purpose.

Stool culture for AFB.—Stool culture for AFB was done in 17 cases of abdominal involvement, so far it has not been possible to grow the bacillus in any case. Out of these 17 cases, the stools of at least eight cases were put up for culture six weeks or more ago. The rest of the cultures are not yet six weeks old and so no definite opinion can be given about them for the present.

Endometrial biopsy.—This examination was done in only married females who were not pregnant. Biopsy was done in 11 cases and put up for histopathology. In none was any evidence of tuberculosis seen. In one case menstrual blood was put up for culture and results are still pending final decision.

Anti-tuberculous treatment taken previously.—Out of the 33 cases who came under investigation, 10 had already taken anti-tubercular treatment in the form of isonicotinic acid hydrazide and PAS. Twenty-three cases had no such treatment before coming to the hospital. According to the different combinations of the anti-tubercular drugs taken, the cases are divided into the following groups:—

Serial number	Group	Number of cases	Streptomycin, g.	Isonicotinic acid hydrazide, g.	PAS, g.
1.	Streptomycin alone ...	6	4 to 20	...	...
2.	Streptomycin and isonicotinic acid hydrazide ...	3	10 to 75	2 to 22½	
3	Streptomycin, isonicotinic acid hydrazide and PAS ...	1	93	16	112

All the three cases with positive culture had taken the anti-tubercular treatment as detailed below:—

One case streptomycin ...	8 g.	
One case streptomycin ...	20 g.	
One case streptomycin ...	75 g.	*plus* 22·5 g. isonicotinic acid hydrazide.

Work done relating to 14 old cases.

Clinical diagnosis.—All the 14 cases were of abdominal tuberculosis.

Age incidence.—The ages of the patients at the start of illness varied from 10 to 32 years and distributed as under:—

Below 10 years	*Nil*
Between 10 and 20 years	6
Between 20 and 30 years	5
Between 30 and 40 years	3

Duration of symptoms.—Before coming to the hospital the patients were having symptoms of disease for a varying length of time. The duration of symptoms varied from 12 days to 6 years and are tabulated as follows:—

Under one month duration ...	2
From one to three months	2
From three to six months	1
From six months to one year ...	1
From one year to two years ...	3
From two years to five years ...	4
Above five years	1

Sex incidence.—Out of fourteen, twelve were females and two males.

Various investigations done.

Sputum and gastric lavage.—Thirteen cases were examined for the purpose. Total number of gastric lavage specimens examined was 25 and that of sputum six. All were negative for AFB.

Mantoux test.—One in 10,000 dilution of old tuberculin was used for the purpose. The test was done in 12 cases and was positive in all.

Radiological investigations.

Lungs.—Skiagram of the chest was done in 12 cases. In eight it showed no abnormality. Amongst the rest, two showed healed lesions, one showed infiltration in the lung fields and one showed enlarged glands.

Barium meal and enema study.—It was done in 12 cases. Ten showed evidence of obstruction of the gut, while two showed no abnormality.

Investigations of the relatives.—The relatives of 11 patients were subjected to screening of their chest. None of them showed any abnormality.

Histopathological examination.—The material for the examination came from glands or intestinal portions removed at operation. It was done in 11 cases. In six cases there was evidence of tubercular pathology, while in five there was no such evidence found.

Culture for AFB.—Out of the 14 specimens which were put up for culture, nine showed no growth of AFB. The growth was obtained in five cases. One mg. of these cultures was injected intraperitoneally in the rabbit and guinea-pig in all the cases. On post-mortem examination no tubercular lesions were detected in any of the animals.

Stool culture for AFB.—Stool culture for AFB was done in eight cases. In none did it show any growth of AFB.

Endometrial biopsy.—It was done in three cases. In none did it show any evidence of tubercular pathology. In one case menstrual blood was put up for culture but it showed no growth.

Anti-tubercular treatment taken previously.—Out of the 14 cases, five had already taken anti-tubercular treatment in the form of streptomycin, PAS and isonicotinic acid hydrazide, before coming to the hospital. According to the different combinations of anti-tubercular drugs taken, the cases are divided into the following groups:—

Serial number	Group	Number of cases	Streptomycin, g.	Isonicotinic acid hydrazide, g.	PAS, g.
1	Streptomycin alone ...	2	20 and 40	...	...
2	Streptomycin with PAS	2	25 and 50	...	50 and 500
3	Streptomycin with isonicotinic acid hydrazide	1	24	12·5	...

Line of work.—In addition to the routine blood, urine, and stool examinations, the following investigations are carried out:—

1. Mantoux test—All cases.
2. Smear and culture of sputum or three specimens of gastric lavage in cases where no sputum is caughed out—All cases.
3. *X*-ray chest—All cases.
4. Barium study (meal and enema)—All cases with abdominal symptomatology.
5. Stool culture—All cases with abdominal symptomatology.
6. Endometrial biopsy—culture and histopathology—Married female cases who are not pregnant.
7. *Biopsy material.*—
   External glands, mesenteric glands, gut after hemicollectomy aspirated material, etc.
   Smear, culture and histopathology is done.
8. Screening of close relatives.
9. Visits to patients' residence to study their relevant residential and environmental factors.
10. Study of source of milk and the condition of the cattle, if any in their houses. Tuberculin test of these cattles will be done by the British double-intradermal test.
11. *Culture.*—The following media are used:—
   Lowenstein-Jensen medium with and without glycerol,
   Dorset egg medium with and without glycerol, and
   Lowenstein-Jensen medium prepared with chicken broth.
12. *Animal inoculation.*—All the specimens which are cultured are also injected intraperitoneally into the guinea-pigs. In cases with negative culture, but positive guinea-pigs results, the tubercle bacillus will be isolated from the guinea-pig lesions by cultures.

24. Inquiry into the antigenic structure of recently isolated strains of *M. tuberculosis* as revealed by the hæmagglutinin absorption technique under Dr. H. I. Jhala at the Grant Medical College, Bombay.

The object of the inquiry was to study the antigenic character of *M. tuberculosis* with a view to ultimate development of a potent antigen for specific sero-diagnosis of tuberculosis, using hæmagglutinin absorption technique. Local strains were to be studied for antigenic character.

It was necessary to obtain 'S' colonial forms to obtain a good specific antigen from each strain. 'S' forms have been reported in the tubercle bacillus by Smithburn (1936) and also by other workers (Petroff *et al.*, 1930, Birkaug, 1933 and 1935, Saenz and Costil, 1934).

As Smithburn's technique is simpler, it was adopted. This consists in inoculating a dilute suspension of tubercle bacillus into three slopes of Corper and Cohn medium, each adjusted to pH 6·0, 6·4, 7·2. Different colonial variants are claimed to grow at different pH.

As the results of the above study became apparent the effect of temperature of inspissation of Lowenstein and Jensen's medium, on the colonial character of the tubercle bacillus, was studied.

Different colonial forms of tubercle bacillus were obtained at different pH on the Corper and Cohn medium and at different degrees of inspissation on Lowenstein and Jensen medium.

MATERIALS AND METHODS

Strains.—Twenty-one strains of *M. tuberculosis* were studied:

Corded.—These were recently insolated on Lowenstein and Jensen medium, from frank tuberculous cases. The organisms are short thin rods, sometimes even coccobacillary, arranged in long serpentine cords—characteristic features of virulent tubercle bacilli. One guinea-pig inoculated with 1 c.c. of ten-day old 'Tween' 80 culture of one of these strains died with gross evidence of tuberculosis, on the 17th day following inoculation. Eighteen strains were studied. Of these seven had been maintained in the laboratory for three generations previous to this study. The other 11 were used directly after primary isolation.

BCG.—Isolated from local BCG vaccine. Organisms are longer often curved and beaded, show slight cording. One strain was studied.

Non-corded.—These were recently isolated from quiescent tuberculous cases. The organisms are considerably longer than the corded strains, curved, beaded, and show no cording. Growth is slower than the corded variants. These are characteristically avirulent tubercle bacilli. Two strains were studied directly after primary culture.

Methods.—A dilute suspension of tubercle bacilli from growth on Lownstein and Jensen's medium was prepared and inoculated into the following media:

(1) *Corper and Cohn.*—At pH 6·0, 6·4, 7·2 to study the effect of pH (Smithburn's technique).

(2) *Lowenstein and Jensen.*—inspissated at

(*a*) 75°C. for ½ hour on two successive days;

(*b*) 80°C. for 1½ hour from the first day and 75°C. for 1½ hour on the second day;

(*c*) 85°C. for ½ hour on two successive days; to study the effect of inspissation.

Media.

1. *Corper and Cohn.*—Prepared according to the instructions of the authors (1933) with the following modifications in the composition:

Egg yolk	100 c.c.
Glycerol (AR)	4 c.c.
Salt solution	33 c.c.

(Na_2HPO_4. anh. 0·2 per cent)

(KH_2PO_4. anh. 0·2 per cent)

2. *Lowenstein and Jensen.*—Prepared according to the instructions given by Mackey and MaCartney (1953) with the following modifications in the composition:

Salt solution:

K_2SO_4 anh.	0·4 g.	Asparagine ...	0·6 g.
$MgSO_4$. $7H_2O$ (AR)	0–04 g.	Glycerol (AR)...	2·0 c.c.

Distilled water to 100 c.c.

Salt solution	300 c.c.
Starch (Litner's soluble) ...	15 g.
Egg fluid	500 c.c.
Two per cent malachite green ...	8 c.c

RESULTS

Different colonial forms were obtained on different media. On any particular medium the colonial morphology remained constant during the period of this study, i.e., one to three generations.

A. *Effect of pH:*

Number of strains studied ...	18 all corded.
Primary culture	Mucoid–like colonies from Lowenstein and Jensen medium (75°C.).
Period of study	3 generations with 7 strains. 2 generations with 11 strains.

Distribution of colonial forms at different pH.

pH 6·0: Colonies are large, flat with irregular edges. Majority show a sharply rising pointed centre. Rough on the surface, strongly adherent to the medium.

pH 6·4: Colonies are rounded or sometimes pointed in the centre with veil like peripheries of varying extent. Rough on the surface, strongly adherent to the medium.

pH 7·2: Colonies are similar to those seen at pH 6·0, but are smaller and thinner, almost translucent and take longer to grow.

No variation in bacterial morphology was noted. Some of these colonies were transferred back to Lowenstein and Jenson medium (75°C.), and immediately regained their original mucoid character. No rough colonies were ever obtained on Lowenstein and Jensen medium (75°C.) or smooth or mucoid colonies on Corper and Cohn medium.

B. *Effect of inspissation:*

(*a*) On corded strains

Number of strains studied	...	10
Period of study ...	...	1 generation.
Primary culture ...	...	Mucoid-like colonies on Lowenstein and Jensen medium (75°C.).

Distribution of colonial forms at different degrees of inspissation.

75°C.—Colonies measure about 3 mm. in diameter, circular, convex with entire edges, smooth, glistening and opaque. Highly resembles the mucoid type of other organisms.

80°C.—Similar to above, but the mucoid character is less developed.

85°C.—Colonies similar to those seen at pH 6·4 on Corper and Cohn medium.

No variation in bacterial morphology was noted. Colony character was found reversible.

(*b*) On the non-corded strains:

Number of strains studied	...	2
Period of study ...	...	1 generation.
Primary culture ...	...	Smooth colonies on Lowenstein and Jensen medium (80°C.).

Distribution of colonial forms at different degrees of inspissation:

80°C.—Colonies are smooth on the surface, rounded considerably raised, and have knob like projections on the surface. Surface is not so glistening as in the mucoid colonies.

85°C.—Colonies are similar to above but are rougher on the surface.

No variation in bacterial morphology was noted. Colony grown on 85°C. medium has not as yet been transferred to medium inspissated at 75°C.

(*C*) Colonial character of different strains on the same medium—Lowenstein and Jensen —80°C.

Corded: Mucoid colonies:

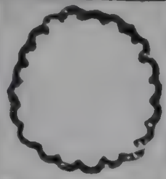
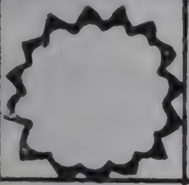
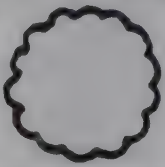
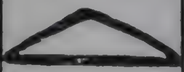
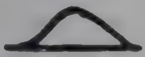
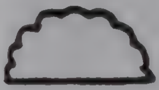
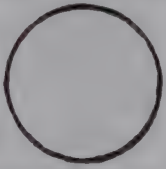
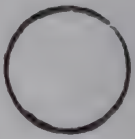
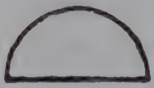
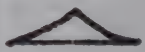
BCG.—Colonies are smooth on the surface, considerably raised and somewhat pointed in the centre. Have knobby projections on the surface. Surface is not so glistening as in the mucoid colonies.

Non-corded.—Similar to BCG but are rounded.

DISCUSSION.

We did not obtain 'S' colony by Smithburn's technique. The results of Smithburn and our work are compared in the Table.

The sudden and dramatic changes in colonial character in Smithburn's type of study appear to us to be an adaptation rather than dissociation for following reasons:—

		OUR RESULTS			
	pH	6·0	6·4	—	7·2
SMITHBURN'S RESULTS	6·0				
					—
		ROUGH	ROUGH	SMOOTH	
	6·4				
		— —	DO		
				SMOOTH	TRANS-LUCENT
	7·2				
		— —	—	DO	DO

(1) The colonial character reverted on transfer to original medium of Lowenstein and Jensen. Such would not be the case in dissociation.

(2) The morphological character of the bacillus is unchanged with reference to size arrangement and cording. One would expect changes in dissociation.

To test whether adaptation occurs, when we grew them on Lowenstein and Jensen medium inspissated at various temperatures. Such media differ in surface wetting characters and yield differing morphological types of colonies by adaptation.

Comparable differences in colonial morphology of the type of those obtained by Smithburn were obtained by us, using corded strains, but we did not get identical results.

Work is being done to promote dissociation in Stenken's modification of Proskauer and Beck liquid medium with media of different pH and latter transferring to a Lowenstein and Jensen medium with standard surface characters. This will give us non-corded avirulent variants.

Our study indicates that ' S ' forms described by Smithburn (*Jour. Exper. Med.*, **63,** p. 95, 1936); Petroff *et al.* (*ibid.*, **51,** p. 831, 1930); Birkaug (*Ann. Inst. Past.*, **54,** p. 195, 1935) and Saenz and Costil (*Compt. Rend. Soc. de Biol.*, **116,** p. 1265, 1934) are in fact identical with corded variants, which are generally considered to be rough and yet virulent. It is proposed to utilize the primary cultures giving rough and virulent (corded) forms for preparing antigens and antisera. To absorb antisera, antigen from avirulent variants obtained by method described in previous paragraph will be used. Absorbed antisera will be used for the hæmagglutination test with antigen from primary cultures.

Six animals have been inoculated for preparation of antisera.

On completion of antigenic analysis by this method the application to diagnostic fields may be possible.

25. Inquiry on the effect of anti-tubercular drugs on the synthesis of phthioic acid in *Mycobacterium tuberculosis* under Dr. S. Chandrasekhar and Lieut.-Colonel A. J. H. de Monte at the Vallabhbhai Patel Chest Institute, Delhi.

A large incubation space is necessary for the above work. This was not available as expected. The work done has, therefore, been negligible. A suitable medium for growing *M. tuberculosis* was found and an attempt to increase the amount of growth/litre was made.

Three liquid synthetic media were tried for submerged culture of *M. tuberculosis* (H37RV), namely Long's, Sauton's and Youman's (without serum). The media were incubated at 37°C. for six weeks. Only Youman's medium gave good growth. This was subsequently used. For isolation of phthioic acid a large amount of bacillary growth is necessary. Earlier workers claimed to have obtained 40 g. to 50 g. wet weight of bacteria per litre of the medium (Metabolism of Tubercle Bacilli-Drea, 1953). Attempts have been made to obtain this. All flasks were washed with concentrated acids and the cotton-wool used for plugs extracted with ether to dissolve traces of fatty acids. Cotton-wool was found to contain traces of fatty acids which are absorbed by the glass during autoclaving and produce adverse effect on the growth of the bacilli.

The inoculum was weighed and ground in a tissue-grinder to disperse the bacilli. 0·02 mg. to 0·1 mg./c.c. is inoculated. Except for once when the growth reached 20 g./litre, the wet weight of bacilli, ranged from 0 to 6 to 3 g./litre. A large number of flasks could not be used for want of incubation space. Therefore, a limited number of experiments only had to be carried out.

26. Inquiry into inter-relationship between tuberculosis and malaria under Dr. (Kumari) H. Patil at the Lady Hardinge Medical College, New Delhi.

All monkeys on first arrival were weighed, tuberculin tested, their hæmatological and stool examination done as a routine. Only tuberculin–ve monkeys were used. Technique of tuberculin test laid down by Nair and Ray (*Ind. Jour. Tuber.* **1,** No. 2, 1954 pp. 1-4) was followed. The strain of tuberculin was the same as used by them. (P. P. D.)

Three groups of experiments were carried out:

GROUP I: Tuberulin–ve monkeys were inoculated with a culture of tubercle bacilli grown on Medler's slope isolated from the sputum of an untreated case of bilateral pulmonary tuberculosis, first isolation, six weeks old culture. The dose used was 0·1 mg. of wet emulsion of the tubercle bacilli per kilogram of body-weight. The routes were intravenous, subcutaneous and intraperitoneal keeping the dose constant. The object was to find the route which gave longest survival time as well as produced discernible tuberculous lesions in the body. The details of the three series are as follows:—

Series (*i*) The intravenous group:
Tuberculin test was repeated on this series ten days after inoculation with tubercle bacilli and hæmatological examination done every week till death.
All of them remained tuberculin–ve. The survival period was between tenth and twenty-seventh day.
The post-mortem findings.—All showed tuberculous lesions in one organ or more.

Series (*ii*) The subcutaneous group :
Site of inoculation was above the right thigh. Hæmatological examination done every week till death. Tuberculin test repeated till it was positive and then they were inoculated intravenously with *P. knowlesi nuri.* They all died after one month and ten days, from the date of inoculation by tubercle bacilli and within five to six days from the day of inoculation by malarial parasites. All showed tuberculous lesion in one or more organs.

Series (*iii*) *The intraperitoneal group.*—Complete hæmatological examinations were done every week till death. Tuberculin test repeated at intervals till it became positive. They were then inoculated with plasm. *Knowlesi nuri* strain intravenously. They all died from six to eight weeks after inoculation of tubercle bacilli and eight days after inoculation with malarial parasites. This group all showed tubercular lesion in one or more organs.

The above experiments are being repeated with smaller doses of tubercle bacilli and malarial parasites.

GROUP II. Experiments consisted of tuberculin–ve monkeys inoculated with plasm. *Knowlesi* 'Nuri strain' in differing doses and routes to find out the most suitable dose for the present inquiry. Eight series of monkeys were inoculated intravenously with eight differing doses and one series intraperitoneally to decide longest period of survival by lessening the number of parasites used as inoculum as a preliminary to subsequent inoculation with tubercle bacilli and vice versa. After inoculation with malarial parasites monkeys were examined twice a day for the appearance (the first positive smear) and degree of parasitism in the peripheral blood and also for observing maximum parasitæmia. They all died with slight increase in survival period in accordance with reduction of dose of malarial parasites. It is proposed to continue this series using only the intraperitoneal route and smaller doses with a view to find out a suitable dose and route for injection in tubercle bacilli inoculated monkeys.

GROUP III. Experiments consisted of tuberculin–ve monkeys inoculated with plasm. *cynomolgi*, in different doses, all intravenous to find out the most suitable dose for the present problem. Four series of monkeys were inoculated with four differing doses to decide the longest period of survival as a preliminary to subsequent inoculation with tubercle bacilli and vice versa. This series is inconclusive. This series is being repeated using super-inoculation with the same strain of malarial parasite.

27. Studies on heart in anæmia and malnutrition under Dr. R. P. Malhotra at the Medical College, Amritsar.

In all nine cases were taken up till 30th September, 1955 out of which one case was discarded as that turned out to be a case of irreversible anæmia due to malignancy. Thus, the present report refers to eight cases in all. Mainly cases of severe to moderate anæmia have been taken up so far, but those of moderate to mild anæmia will also be studied in future.

Age incidence.

The ages of the patients under study varied from 18 years to 35 years and distribution as follows:—

Between 10 and 20 years	2
Between 20 and 30 years	3
Between 30 and 35 years	3

Sex incidence.

Out of eight cases, one was female and the rest were males.

Diet and economic status.

Out of eight cases, the economic condition of two cases was quite poor with poor diet, five cases had just average economic condition with borderline diet and one case had a good economic condition with a good nourishing diet. Two are vegetarians and six are non-vegetarians.

Type of work.

Hard manual labour	4
Moderate labour	2
Sedentary labour	2

Chief symptoms.

Breathlessness on exertion	7
Palpitation on exertion	6
Generalized weakness and fatigue	6
Headache and giddiness	3
Burning sensation hands and feet	3
Diarrhœa	3
Insomnia	2
Irregular fever	2
Visual disturbance	1

Cause of anæmia.

Ankylostomiasis	3
Chronic diarrhœa	1
Chronic diarrhœa with chronic malaria ...	1
Chronic diarrhœa with chronic malaria and ankylostomiasis	2
Ankylostomiasis and internal piles ...	1

Severity of anæmia.

Hæmoglobin level below 5 g.	5
Hæmoglobin between 5 to 7 g.	1
Hæmoglobin between 7 to 9 g.	2

Line of work.

The various investigations which have been and are being carried out and repeated at suitable intervals in addition to the clinical study, are given below:—

1. Complete hæmogram (hæmoglobins and red blood cell repeated at weekly intervals).
2. Urine.
3. Stools.
4. Gastric analysis.
5. Blood proteins.
6. Bone marrow biopsy (where indicated).
7. Electrocardiographic studies.
8. Radiological studies.
9. Venous pressure.
10. Circulation time (both arm to lung and arm to tongue time).
11. Other relevant investigations which may be required in a particular case.

28. Inquiry on the clinical application of electro-retinography in cataract, glaucoma and detachment of retina under Dr. R. P. Dhanda at the M. G. M. Medical College, Indore.

Clinical applications of electro-retinography not only to different aspects of ophthalmology, but even in conditions, such as arterial hypertension in human beings have been reported in international medical literature in recent months, specially in U.S.A., Sweden and Netherlands.

The object of the present work is:—

(1) To determine by electro-retinography, the grade of disturbance caused to the eye and more specially to retina in different methods of cataract extraction.

(2) To assess the prognosis of a particular eye by observing the b-potential before operation, because ophthalmoscopy is not possible and reliance only on the presence of PL and PR is an uncertain guide. Electro-retinography may, therefore, be a precise means of assessing the prognostic value.

(3) Similarly, study of cases of glaucoma by electro-retinography as reported in international literature has prompted me to do further investigations.

(4) Detachment of retina as a subject of investigations by electro-retinography is another similar item.

·32mV as the average b-potential of a normal Indian adult determined on the basis of investigations of nearly 100 *normals studied at this electro-retinography unit during the last three years is taken as the standard normal b-potential for this subject of study.*

CLINICAL INVESTIGATIONS.

Cases of cataract.—Sixteen cases of different types of senile cataract were investigated during the first five months of this year. Complete history and objective findings were recorded in each case and electro-retinogram taken 24 hours before the date of operation. The operation performed was deliberately decided intra-capsular in some and extra-capsular in others. Post-operative findings were noted and second electro-retinogram was recorded between the tenth and fifteenth post-operative day depending upon the condition of the eye. Third electro-retinogram was repeated in one case who turned up for prescription of glasses eight weeks after the operation.

It is proposed that further electro-retinograms should be repeated in as many of these cases as possible even up to two to three years after the operation because it has been suggested that the disturbance to the retina during the immediate post-operative period may not be enough to disturb the b-potential at this stage but the same which may progress and ultimately initiate a remote complications, such as detachment of retina may demonstrably affect the b-potential.

The average b-potential of 13 eyes with mature cataract was **0·252 mV.** and in 16 eyes with immature cataract the average b-potential was **0·292 mV.**

TABLE I

Showing different types of senile cataract indicating b-potential in mV. in different eyes before operation.

Name and age		Mature cataract	Hyper mature cataract	Immature cataract
G.	65	0·200	...	0·250
G.S.	65	0·290	...	0·280
K.B.	70	0·282	...	0·260
L.B.	65	...	0·28	0·260
Ch.B.	60	0·190	...	0·311
P.R.	45	0·217	...	...
G.L.	45	0·175	...	0·212
M.H.	60	0·210	...	0·307
G.	60	...	0·27	...
B.B.	60	...	...	0·350
W.K.	65	0·233	...	...
R.H.	55	0·300	...	0·309
B.B.	72	0·225	...	0·225
L.B.	45	0·425	...	0·421
K.B.	35	0·275	...	
D.P.	35	0·243	...	
		0·300	...	0·310

This suggests that the b-potential is to some extent reduced by the formation of cataract but it also proves that a good amount of electric potential can be recorded even in cases of mature cataract where the vision is reduced to hardly hand movements. This implies that even a very small amount of light passing through a nearly opaque lens and the spongy iris is enough to bring the retina to appreciable activity. One could conclude from this that retina behaves more or less on the lines of ' all or none phenomenon '.

Electro-retinograms have been repeated after the operations in 11 cases but it is too early to reach any conclusions at this stage, which is only possible after the work is continued for a few years. One observation which may be mentioned is that there is a distinct increase in b-potential in the immediate post-operative period but what happens later, is yet to be observed.

Table II gives the summary of observations.

Cases of glaucoma.—Eleven eyes of nine cases of glaucoma were investigated during this period of which three eyes had acute type of glaucoma and two eyes of absolute glaucoma. Six eyes had chronic glaucoma with some amount of vision. In three of these cases electro-retinogram was repeated after trephining operation while in the fourth case electro-retinogram was repeated after Diamox therapy.

Here again the number of cases and the period of investigations is too short for substantive conclusions.

One thing that may be specially mentioned that a good amount of electric response may be present even in an eye with a very high tension

TABLE II

Number.	Name	Age and sex		Diagnosis	E.R.G. before operation on	b-pot.	in mV.	Type of operation	E.r.g. after operation on	b-pot.	in mV.	Remarks
1	G.	65	Male	R.E. Mat. Cat. L.E. Im. Cat.	7–1–1955	R.E. L.E.	0·2 mV. 0·25 mV.	R. E. Intra-capsular	21–1–1955 27–5–1955	R.E. L.E. R.E. L.E.	0·39 mV. 0·206 mV. 0·3 mV. 0·25 mV.	
2	G.S.	65	Male	R.E. Mat. Cat. L.E. Im. Cat.	27–1–1955	R.E. L.E.	0·290 mV. 0·280 mV.	R. E. Intra-capsular	4–2–1955	R.E.	0·333 mV.	
3	K.B.	70	Male	R.E. Im. Cat. more Mat. in R.E.	2–7–1955	R.E. L.E.	0·260 mV. 0·282 mV.	R. E. Intra-capsular	18–7–1955	R.E.	0·3 mV.	
4	L.B.	65	Female	R.E. Hyper Mat. Cat.	14–7–1955	R.E. L.E.	0·28 mV. 0·26 mV.	R. E. Extra-capsular	23–7–1955	R.E.	0·200 mV.	
5	G.B.	60	Female	R.E. Mat. Cat. L.E. Im. Cat.	28–4–1955	R.E. L.E.	0·190 mV. 0·311 mV.	R. E. Extra-capsular	18–8–1955	R.E.	0·204 mV.	
6	P.R.	45	Male	R.E. Mat. Cat. L.E. Aphakia.	18–2–1955	R.E. L.E.	0·217 mV. 0·230 mV.	R. E. Extra-capsular	28–2–1955	R.E.	0·495 mV.	
7	G.	45	Male	R.E. Mat. Cat. L.E. Im. Cat.	18–5–1955	R.E. L.E.	0·175 mV. 0·212 mV.	R. E. Intra-capsular	28–7–1955	R.E. L.E.	0·253 mV. 0·223 mV.	
8	M.H.	60	Male	R.E. L.E. Mat. Cat.	6–5–1955	R.E. L.E.	0·307 mV. 0·210 mV.	L. E. Extra-capsular	16–6–1955	L.E.	0·433 mV.	
9	G.	60	Male	R.E. Sec. Gla. L.E. Hyper Mat. Cat. Sub. lux.	5–8–1955	R.E. L.E.	0·240 mV. 0·27 mV.	R. E. Intra-capsular	17–8–1955	R.E.	0·29 mV.	
10	B.B.	72	Female	R.E. Im. Cat. L.E. Mat. Cat.	21–7–1955	R.E. L.E.	0·225 mV. 0·225 mV.	L. E. Extra-capsular	28–7–1955	L.E.	0·250 mV.	
11	W.	65	Male	R.E. Abs. Gl. L.E. Mat. Cat.	13–8–1955	R.E. L.E.	 0·233 mV.	L. E. Extra-capsular				
12	B.B.	60	Female	R.E. Mat. Cat. L.E. Im. Mat. Cat.	28–7–1955	R.E. L.E.	0·35 mV. 0·356 mV.	R. E. Extra-capsular				
13	D.P.	35	Male	R.E. L.E. Mat. Cat.	25–2–1955	R.E. L.E.	0·295 mV. 0·243 mV.	L. E. Extra-capsular				
14	R.N.H.	55	Male	R.E. Mat. Cat. L.E. Im. Mat.	18–2–1955	R.E. L.E.	0·30 mV. 0·309 mV.	R. E. Extra-capsular				
15	L.B.	45	Female	R.E. Im. Cat. L.E. Mat. Cat.	24–5–1955	R.E. L.E.	0·421 mV. 0·425 mV.					
16	K.B.	35	Female	R.E. Mat. Cat. L.E.	25–2–1955	R.E. L.E.	0·275 mV. 0·26 mV.	R. E. Intra-capsular				

and very poor vision. This can be explained by the possibility of an electrical potential developing in those layers of retina which do not suffer from the effects of high tension.

Electro-retinography should be continued in different types of glaucoma for at least two to three years before the conclusions from the data can be usefully recorded.

Detachment of retina.—Only three cases of detachment of retina were observed during this period. In all of them the electric potential was sub-normal indicating poor prognosis. Of course these cases had a total detachment and, therefore, were unfit for operation.

Electro-retinographic studies to be of useful value should be done in cases of partial detachment which are available only at long intervals and, therefore, the work should again be continued for three more years.

29. Biochemical and serological investigations on Escherichia Klebsiella strains isolated from urinary infections under Dr. S. P. Gupta and Dr. N. P. Gupta at the King George's Medical College, Lucknow.

Three hundred and four strains of organisms of this group were isolated from cases of urinary infection in this department. Strains isolated from urine showing significant number of pus cells in urine were only included in this investigation.

They were subjected to the following biochemical investigations:

(*i*) Fermentation of sugars: lactose, glucose, sucrose, local mannitol, maltose, adonitol, inositol, dulcitol, sorbitol, xylose, arabinose, rhamnose and salicin. Reaction was observed with these sugars in peptone-sugar media for ten days.

(*ii*) Fermentation of organic acids: d-tartrate, sodium citrate and sodium mucate. These were observed for six days.

(*iii*) Decomposition of urea, i.e., urease activity: This was observed as a routine for six days using Christensen's urea agar medium.

(*iv*) Production of indole: Organism was grown in peptone water culture and test was done on fourth day.

(*v*) Methyl red and Voges Proskauer reaction: Organisms were grown in glucose—phosphate medium and were tested on the fourth day of incubation.

(*vi*) H_2S production and gelatin liquefaction: Ferrous chloride gelatin media was used and was observed for six days.

(*vii*) Nitrate reduction was tested on fifth day after growing the organism in nitrate broth.

(*viii*) Hæmolysis test was done using sheep's erythrocytes following the technique of Greig.

(*ix*) Hemagglutination test was done using guinea-pig, sheep and human red cells.

Serological studies:

Twenty-five O-groups sera were raised by following the technique of Kauffmann (1947). Standard strains used as antigen were obtained from Serum Institute, Copenhagen.

Preparation of high titre sera.—Rabbits between 1 kg. and 1½ kg. in weight were selected and tested for α and β antibodies with the available antigens. Antigens for injection into the rabbit were prepared by culturing the organisms in nutrient agar for 20 hours and then suspended in normal saline. The suspensions in saline were heated at 100°C. for 2½ hours to destroy the L, A, B and H antigens of *Escherichia coli.* They were then diluted to contain 1,000 million organisms per c.c. This was injected in doses of ·25 c.c., 0·5 c.c., ·5 c.c., 1·0 c.c., 1·0 c.c. intravenously on every fourth day. Seven days after the last injection sera were collected from the ear veins and titrated. If found satisfactory blood was collected and serum preserved in 50 per cent neutral glycerine.

Preparation of antigen for agglutination reaction.—The organisms were grown in thick soft agar for about 24 hours and suspended in saline and heated at 100°C. for one hour. Then for slide agglutination technique it was diluted to contain 10,000 million organisms per c.c. and for tube agglutination suspension contained 1,000 million organisms per c.c.

Results.

(*a*) *Biochemical.*—After studying various correlated characters the best way to classify these organisms was found to be based on the following biochemical characters:—

	Escherichia	*Klebsiella*
Adonitol	—	+
Inositol	—	+
M.R.	+	—
V.P.	—	+
Indole	+	—
Urea	—	+
Citrate	—	+
Motility	+	—

According to these characters 213 strains were found to be *Escherichia* and 86 strains were found to be *Klebsiella*, while 5 strains remained unclassified. No single character was specific for any group and all the characters had to be considered for the classification.

(*b*) *Serological.*—Out of 213 *Escherichia* strains, 100 strains were tested by both slide and tube agglutination technique and another 50 strains have been tested with slide-agglutination technique alone. Results with slide and tube agglutination were found to be parallel in 100 strains.

O-groups	By slide agglutination technique	By tube agglutination technique
O-group 3	4 strains	4 strains
O-group 4	2 ,,	2 ,,
O-group 6	5 ,,	5 ,,
O-group 7	2 ,,	2 ,,
O-group 8	1 ,,	1 ,,
O-group 9	2 ,,	2 ,,
O-group 11	5 ,,	5 ,,
O-group 15	16 ,,	16 ,,
O-group 16	3 ,,	3 ,,
O-group 17	3 ,,	3 ,,
O-group 20	4 ,,	4 ,,

Thus, 47 percent of the *Escherichia* strains could be serologically typed with these 25 O-group sera.

Another 50 strains were typed serologically by slide agglutination only. The results obtained so far with these 150 strains may be tabulated as follows::

Table showing result of serological classification of 150 strains of *Escherichia coli.*

O-groups	Number of strains found
O-group 3	5
O-group 4	4
O-group 6	12
O-group 7	2
O-group 8	1
O-group 9	2
O-group 11	6
O-group 15	22
O-group 16	3
O-group 17	6
O-group 20	7
Unclassified	80
Total	150

This shows that 46·2 per cent of the organisms could be placed within the 25 O-group of the Kauffmann, Knipschild, Valne diagnostic scheme.

30. Investigation into the rôle of allergens and various other factors in the production of bronchial asthma in Rajasthan in general and in Jaipur area in particular under Dr. R. M. Kasliwal at the S.M.S Medical College, Jaipur.

Synopsis.—The pollination calendar of a place is a record of the allergenic plants of the time in days and months when they pollinate and attain sufficient concentration in the atmosphere to cause allergy. It will be realized that the first step towards distinguishing local allergenic plants is to obtain a thorough knowledge of the local vegetation as well as of the time when they effloresce. It is also equally clear that the problem is as much botanical as medical.

To investigate the problem the work has been planned as under:—

1. Botanical survey of the area as related to the pollination calendar.
2. Study of the atmospheric pollens and their correlation with botanical survey.
3. Seasonal collection of pollens and preparation of their extracts.
4. Determination of allergenic pollens by performing skin tests with their extracts.
5. Charting of the allergenic pollens according to the time of pollination.

Botanical survey of the area.—The botanical survey of this area has not been done before. In order, therefore, to know the local flora the first task was to do the field study of the plants.

Before proceeding on a botanical survey, it was considered essential to know the names of the plants which may be allergenic, *i.e.*, whose pollens are anemophilous or are produced in very large quantity. On perusing the botanical literature (Duthie, Thakur, Blatter, McCane) and with the help of local botanists a list could be prepared of such plants. The list is given below:

Gymnosperms :—They usually grow in the hilly tracts. The plant which grows here abundantly and may be of importance is Thuja, sometimes called Mayur Pankhi.

Angiosperms :—Most of the important anemophilous plants belong to this group. Even though divided into monocots and dicots, former is by far the commonest source of allergenic plants. In the monocots graminæ are the most important and of this following are important members which grow here.

Grasses :—

Sorghum halepense
Dichanthium annulatum
Digitaria marginata
Digitera pennata
Heteropogan contortus
Themeda triandra
Panicum crusgalli
Panicum antidotale
Setaria italica
Setaria glauca
Pennisetum
Cenchrus catharticus
Cenchrus biflorus
Cenchrus ciliare
Aristida adscensionsis
Aristida funiculata
Aristida mutabilis/
Sporobolus diander
Eragrostis teremula
Eragrostis tenella
Eragrostis pilosa
Eragrostis ciliaris
Cynodon dactylon
Chloris gayane
Elusive indica
Saccharum spontaneum
Saccharum munja
Gracilea royleana

Of the agricultural grasses the following are the important ones—

Wheat
Barley
Bajra
Jwar
Maize
Oat

Other plants of graminæ series are listed below:—

Cyperacæ —*Cyperus rotundus*
Frimbistlis
Scirpus martimus

Typhacæ —*Typha*
Palmæ —*Phæniz*
Borassus

Of the anemophilous dicots no plant belonging to walnut, willow, birch, beech, elm, hamemelidus or maple family grow in this area. Other dicots which may be important from the point of allergy are given in the list below:—

Mulberry —*Morus alba*
Morus indica
Urticacæ —*Holoptelea integrifolia*
Amaranthacæ—*Amaranthus spinosus*
Amaranthus gargeticus
Amaranthus blitum
Amaranthus polygamus
Amaranthus viridis
Aerua tomentosa
Achyranthes aspera
Pupalia lappaceæ
Digera arvensis
Celosia argentea
Rosacæ —*Rose*

Cæsalpnoidæ —*Cassia*
Bauhinia
Delonise
Tiliacæ —*Corchorus*
Grewia asiatica

Chenopodiacæ —*Kochia*
Atriplex

Leguminosæ —*Mimosæ*
Acacia jacquemontii
Acacia arabica
Acacia sinegul
Acacia catechu
Acacia leucophlæa
Acacia farnesiana
Albizzia lebbek
Albizzia odoratissima
Prosopis spicigera
Prosopis juliflora
Inga
Scitaminæ —*Plantain*
Compositæ —*Artemesia*
Aster
Chrysanthemum
Daizy

For purposes of botanical survey, area around Jaipur was selected. Though it is possible that the pollens may travel a distance of as many as 18 miles (Tuft) , or rarely as much as 400 miles (Vanghan and Black), yet as a first programme the area within a radius of five miles was selected. Special attention was given to those areas where grasses and weeds grow in abundance. This included the foot of the mountain of the Aravali range, and the old and new ghats where foliage is abundant. Certain protected areas within the town, and the gardens and fields where agricultural products are growing were also investigated.

A survey of these areas was made twice a week, and mostly the evening hours were slected for collection of the plants and their pollens.

Most of the plants were identified on the spot, as one of the botanists kindly accompanied us. In other cases the plant was collected and preserved under layers of blotting papers and recognized later on. The possible allergenic plants which blossom during the months between April and September are given in the following list:—

Leacas aspera	...	April
Bauhinia	...	April
Albizzia lebbek	...	April and May
Acacia arabica	...	April and May
Acacia jacquemontii	...	April and May
Prosopis spicigera	...	April and May
Euphorbia dracuncauoides	...	April, May and June
Melia azadirachta	...	April and May
Acacia catechu	...	May to July
Grewia asiatica	...	June to August
Sorghun halepense	...	all year round
Eragrostis tremula	...	all year round
Maize	...	September to ———
Jwar	...	September to ———
Bajra	...	Spetember to ———
Ailanthus excelsa	...	March and April
Cynodon dactylon	...	all year round
Solanum xanthocarpum	...	all year round
Guava	...	May and June
Mehandi	...	June, July, August.
Poinciana regia	...	April and May

It will be seen from the above list that it includes mostly the trees and very few grasses. It is because this year the rains have been delayed here by a month and a half, and once started they are continuing. The vegetative phase of the grasses is well on the way, but their reproductive phase has not yet started. Considering that in Bombay area Blattr and Bhide reported many hundreds of grasses, it is felt that in this area also grasses will form the bulk of material to be collected and tested.

The season of weeds started after September, and usually they cause allergy during the months of October, November and December. Their collection has been deferred till they pollinate.

Study of field and ærial pollens.—The ultimate aim of the present study is to know the allergenic pollens which may be present in the atmosphere in any season.

In order, however, to recognize the ærial pollens a field study of them is essential. The pollens of all plants that have flowered between April and September have been collected, studied and their diagrams made. This would serve as reference for atmospheric pollen study.

The slides to catch the atmospheric pollens are exposed on the roof roof of S.M.S. Hospital. This place has been selected because of the ease of approach and because it lies on the verge of vegetation. The roof is about 80 feet above the ground level and has a free display of air current in all directions. A paraffin coated microscopic slide is placed in the device at 10 a.m. and removed after 24 hours. Slide is examined for pollens under the microscope. Caberla's fluid is used for staining. A daily record is made of the type of pollens encountered, as well as of the number per cover slip area.

In the arid months of April, May and June the count per cover slip area varied from 0 to 4 per 24 hours while in July, August and September it has steadily increased and now the count varied from 10 to 50. Most of the pollens appear to be coming from grasses. Identification of the exact source of pollens is not only difficult, but impossible at the present stage of study.

Seasonal collection of pollens and preparation of their extracts.—The pollens of all those plants which have been given in the 2nd list have been collected. The collection was made on the spot where the plants grow and the technique as described by Williams was used. The time of collection of pollens varied accordingly to the best period for efflorescene; in some plants early hours of the morning were the best, while in others the efflorescence was maximum only after the sun had shown over them for some time.

The pollens are sprinkled direct in a Petri-dish, and about a test-tube full of them are stored in a refrigerator till extracted. Extract of pollens is made as described by Tuft. Powdered pollens is washed twice with water-free ether. The pollen settles quickly in the ether after mixing; following which ether is decanted. The ether is allowed to evaporate by exposure to the air. The sediment is then ready for extraction. It is then weighed and treated with Coca's extracting fluid (NaCl 5 g., $NaHCO_3$ 275 g., phenol ·4 g. distilled water 100 c.c.) in the proportion of 3 g. to 10 g. to 100 c.c. of fluid. This is added to the pollen and shaken gently. It is then allowed to stand for 18 to 24 hours, being stirred up several times during the period. The liquid is decanted and sterilized by filteration.

Most of the pollens collected so far have been extracted. The extracts are stored in a referigerator till required for testing.

31. Inquiry on suprarenal activity in experimental cholera under Dr. S. N. De at the Nilratan Sarkar Medical College, Calcutta.

(1) *Intravenous injection of different doses of* V. choleræ *emulsion.*—Different doses (0·2 c.c. to 1·0 c.c) of very thin to heavy emulsion of 24-hour agar culture in normal saline of *V. choleræ* were injected intravenously into adult rabbits. No diarrhœa or death followed in any instance. Pure culture of *V. choleræ* was obtained constantly from the gall bladder bile even to 18th day. Sometimes a few colonies were also obtained from the spleen or small intestine.

(2) *Injection of different doses into the upper part of the jejunum with injection of tinct. opii.*—Robert Koch's guinea-pig experiments were repeated on rabbits given tinct. opii. Instead of oral introduction of *V. choleræ* after large amounts of alkali, the vibrio was injected into the duodenum after laparotomy as 1.c.c. of 24 hours' culture in pH 8·4. peptone-water medium. Animals did not die in 48 hours nor any symptoms were produced. Vibrios survived in gall bladder bile (pure culture) and small intestines in diminishing number downwards. No excess of fluid was seen to accumulate here. When very heavy dose was used, e.g., emulsion of a whole agar culture, the animals died in 24 hours with no diarrhœa. Experiments are to be repeated with intermediate doses.

(3) *Injection of different doses into jejunum after ligature to stop entry of acid chyme.*—Almost similar experiments with the smaller dose were performed after tying the duodenum to prevent entry of acid gastric juice. The animals died before 24 hours with no diarrhœa.

32. Electrophoretic studies of normal and pathological human sera under Shri N. C. Datta at the Grant Medical College, Bombay.

Introduction .—In recent years much work has been done to correlate the results obtained from salting out with those from electrophoresis. Howe's method of fractionation of serum protein which is commonly used for routine clincial analysis gives values of albumin higher than obtained by electrophoresis. Majoor (1949) reported that euglobulin precipitation with 18·5 per cent sodium sulphate corresponds fairly well with electrophoretic gamma globulin.

Investigation carried out.—Analysis of serum proteins were carried out both by paper electrophoresis and also by chemical fractionation using 26·8 per cent and 18·5 per cent sodium sulphate (for precipitation of globulin and euglobulin, respectively) in 15 cases of nutritional œdema and in 10 cases of cirrhosis of the liver. Average values of albumin, globulin, euglobulin and gamma of different fractions are listed in Table I.—

TABLE I.

Number of cases	Condition	Method employed	AVERAGE VALUE OF			
			Albumin, g. per cent	Total	Globulin euglobin	Gamma
7	Nutritional œdema	Howe	2·32	2·77	1·77	—
7	,, ,,	Majoor's	1·76	3·44	2·27	—
7	,, ,,	Electrophoresis	1·88	3·69	—	2·30
10	Cirrhosis	Majoor's	2·42	3·98	2·69	
10	,,	Electrophoresis	2·30	4·03	—	2·74

From the results of Table I it is evident that there is close agreement between the value of albumin and globulin obtained by electrophoresis when compared with those by chemical fractionation using 26·8 per cent sulphate. The values of euglobulin are in perfect agreement with those of electrophoretic gamma globulin.

The results of electrophoretic analysis of serum albumin in 15 cases of nutritional œdema and in 10 cases of the liver with ascites and positive liver function tests are listed in Table II. For comparison normal values are included.

TABLE II.

Number of cases	Albumin	Total globulin	Alphal 1 per cent	Alphal 2	Beta	Gamma	Authority
Normal sera with normal hepatic function.							
10	4·23	2·78	0·28	0·68	0·89	0·93	Sterling *et al.*
—	4·04	2·34	0·31	0·48	0·81	0·74	Dolel
3	4·84	4·50	2·29	0·25	0·66	0·96	Present series
Nutritional œdema with positive liver functions.							
15	1·88	3·69	0·28	0·41	0·80	2·30	Present series
Cirrhosis of liver with ascites and positive liver function.							
10	2·30	4·03	0·19	0·47	0·63	2·75	Present series
*1	2·56	4·17	0·28	0·43	0·85	2·61	

*1 case estimated by original Tselius apparatus (Sterling *et al.*, 1949).

Electrophoretic analysis of serum protein in 15 cases of nutritional œdema and 10 cases of cirrhosis of the liver revealed deviations for the normal. The albumin fractions were adiminshed in all, and the gamma globulin elevated in all. Elevated beta globulin were observed in 4 of the 15 cases in nutritional œdema and only in 1 case of cirrhosis of the liver.

The results of analysis of serum protein in 10 cases of cirrhosis agree with the value reported by Sterling in 1 case, in which the estimation was carried by original Tselius apparatus. The case of portal cirrhosis differs from billiary cirrhosis in that beta globulin was reported to be increased in 7 out of 10 cases reported by Sterling *et al.* (1949).

Attempts have been made to correlate the results of different protein fractions with the various liver function tests, such as cephalin-flocculation, thymol turbidity and takata-ara tests. The occurrence of positive cephalin-cholesterol flocculation gamma globulin, but there was no strict correlation. This is unexpected in view of the complexity of the reactions.

It is proposed to follow the cases of cirrhosis with electrophoretic analysis of serum porteins. Attempt is also being made to develop the method of *qualitative* study of serum spectrum as described by Buttle (*Jour. Royal Soc. Med.*, 1954).

33. Protective and adrenergic effects of administered amino-acid methionine, glycine, and glutamic acid under Dr. Shiv Kumar at the Medical College, Amritsar.

Both biochemical and histochemical work on the enquiry was started in April 1955. Since the last report (submitted in September 1955), the method for histochemical demonstration of succinic dehydrogenase has been standardized for this laboratory, namely, the modification of Rosa and Velardo (1954). In the preliminary experiments the presence of succinic dehydrogenase activity in the islet tissue of the pancreas, utilizing the reduction of neotetrazolium by succinic dehydrogenase into an insoluble blue formazan dye, has been demonstrated. And it has been concluded as a result of our preliminary observations, that the islet tissue exhibits succinic dehydrogenase activity and its activity in the islet tissue is much lower than that of the acinar tissue. This is in conformity with the results of Lazarow and Cooperstein (1951).

The work is in progress according to the modified plan, and the following experiments are in progress :—

1. In the controls a weekly check is being made on the blood glutathione and sugar.
2. In the second group, viz., *control plus amino acid*, the amino acid (glutamic acid, glycine or methionine) is administered by a stomach tube (2·5 c.c. of a 5 per cent solution per 100 g. body-weight) once a day. The blood estimations are repeated at suitable intervals.
3. In the third group, viz., *control plus stress*, alloxan is being employed as the stresser (a single diabetogenic dose, intravenously). Blood estimations are repeated at suitable intervals.
4. In the fourth group, 2 sub-groups are being studied:—
    - (*a*) *Control plus amino acid plus stress* (the stress and the amino acid being given simultaneously); the rest of the experiment as under 3.
    - (*b*) *Control plus amino acid for one week plus stress* (amino acid is continued even after the application of stress); the rest of the experiment is as under 3.

The histochemical study of the pancreas, kidney and liver is made at suitable intervals by sacrificing the animals, and on post-mortem in addition to the body-weight, the kidney, the supra-renal and the liver are weighed. When sufficient data have accumulated, it will be subjected to a statistical analysis and conclusions drawn.

34. Histochemical investigations on neuro-humoral transmission under Dr. C. M. Francis at the Medical College, Trivandrum.

Previously, it had been shown that there are some cells in the adrenal medulla which are true ganglion cells. These ganglion cells contain amine oxidase and hence they are adrenergic neurons. It had been supposed by most workers that the preganglionic fibres are cholinergic. If that is so, then cholinesterase must be found at the endings of the preganglionic fibres.

Since the work on amine oxidase had been done on guinea-pig adrenal medulla, the same species of animal was used. Frozen sections (25 microns) of the fresh adrenal gland of guinea-pig were prepared. Cholinestrase was localized in the sections by the method of Koelle (1951) with maleate-tris (hydroxymethyl) aminomethane as buffer. Deposits of copper sulphide indicating the presence and activity of cholinestrase were seen on the surface of the ganglion cells in the medulla, i.e., at the terminations or synaptic endings of the preganglionic fibres. Transmission of impulses across these injections must then be mediated by acetylcholine. No cholinestrase was demonstrable along the fibres themselves.

Feldberg *et al.* (1951) had postulated that there is alternation of cholinergic and non-cholinergic fibres. In the visual pathway, the bipolar cells are cholinergic. If the hypothesis of Feldberg *et al.* (*loc cit.*) is correct, then, the optic nerve fibres will not be cholinergic and no cholinestrase will be found at their terminations in the visual cortex. Attempt was made to localize cholinestrase in the optic cortex and cholinestrase has been found at the terminations of the optic nerve fibres in the cortex. Analysis of the fibres and terminations have not, however, been complete and the results and conclusions are only tentative and the work is being continued.

35. Inquiry on 'Higher nervous control over visceral activity' under Dr. B. K. Anand at the Lady Hardinge Medical College, New Delhi.

This inquiry was conducted last year under the heading of "Stimulation of the 'visceral brain' with special reference to blood pressure and respiration ", and the work has been continued this year under the inquiry named above. A note on the results obtained during the first six months was submitted last year.

Stimulations of the different brain structures belonging to the limbic system (which have been collectively named the 'visceral brain', as they control the visceral activities), have been completed. Work now has been undertaken to study the effects of surgical, as well as electrolytic lesions, in these different regions, on certain visceral activities.

I. *Electrical stimulation of the limbic system of brain 'visceral brain' in the waking animals.*

Phylogenetic and cytoarchitectural studies, together with recent physiological investigations, suggested that the limbic system surrounding the hilus of the brain, and comprising the orbital surface of frontal lobes, the anterior cingulate gyrus, the temporal polar area, the pyriform cortex, the hippocampal and dentate gyri; and the associated subcortical cell stations belonging to the amygdaloid nuclei, and the hippocampus, represent an early neural development involved in the higher control of autonomic nervous system and in the effectively determined behaviour. It has a marked influence over the viscera controlled by the autonomic system, hence the designation 'visceral brain'.

In 29 cats and 13 monkeys (*macacus*), multilead electrodes were permanently implanted in both cerebral hemispheres, in different parts of the limbic system. A week after recovery these areas were electrically stimulated in unanæsthetized and waking animals and the effects of such stimulations noted. The parameters of stimulations used were 30/sec. square wave pulses of 0·2 to 1 msec. duration and 3 to 8 volts intensity. In the end the carotid artery and trachea of these animals were cannulated and the blood pressure and respiration recorded before and during stimulation. The animals were then sacrificed and electrode positions confirmed histologically.

(1) *Affective behaviour* of the animals showed marked and varied changes, on stimulation of different regions. Temporal lobe structures (amygdaloid nuclei, both anteromedial and basolateral groups; hippocampal formation; pyriform cortex), excluding the tip made majority of the animals agitated and fearful, and quietened some of them. Temporal tip stimulation in cats made them very irritable. Stimulation of temporal tip in monkeys and posterior orbital cortex just rostral to temporal tip in cats, made them very vicious and violent. On the other hand, cats with electrode locations further removed from temporal tip, as well as monkeys, became very quiet during posterior orbital stimulation. Stimulation of the anterior cingulate made the animals very vicious and violent, and in cats also produced convulsions with intensities ordinarily employed, followed by a typical rage reaction.

(2) *Somatic movements.*—Certain movements involving the ipsilateral facial, eye-lid, orbital, and oral muscles, and rarely limb muscles, and dorsal retraction of head were obtained from all these regions. Contraversive turning of head was only obtained in some cats from amygdala, orbital cortex, and cingulate gyrus.

(3) *Eating responses.*—Organized movements of ‘ eating ’ automatisms (such as sniffing, licking, chewing, biting, chop-licking) were also obtained from all the regions. These were usually accompanied by profuse salivation. But the daily food intake was neither increased nor decreased by any of these stimulations.

(4) *Autonomic activity.*—Stimulation of widespread points in all these structures produced signs of such activity, including lachrymation, salivation, pupillary changes, movements of nictitating membrane, and more rarely urination and defæcation.

The detailed results have been published as under :—

(*i*) Stimulation of limbic system of brain in waking animals. *Science*, 1955, **122,** p. 1139.

(*ii*) Electrical stimulation of the limbic system of brain (‘visceral brain’) in the waking animals. *Ind. Jour. Med. Res.*, 1956, **44,** pp. 107-119.

(5) *Blood pressure.*—Changes were elicited on stimulation of all the different regions. Stimulation of temporal lobe structures excluding the tip, produced a fall in B.P. in the majority of cats and monkeys. Temporal tip stimulation in monkeys produced a fall in B.P., while in cats this produced a rise. The frontal lobe structures, orbital cortex and cingulate gyrus, generally produced a rise in B.P.

(6) *Heart rate* on the other hand, was both increased, as well as decreased, on stimulation of all these regions. There was no correlation between the changes in heart rate and B.P.

(7) *Respiration.*—Changes in respiration could not be produced from all the points stimulated, and there was no correlation observed between the changes in respiration and B.P. There was usually an inhibition of respiration (even apnœa in expiration in some) on stimulation of temporal lobe structures, and usually an increase in both depth and rate on stimulation of frontal lobe regions. But opposite effects were also observed. These results have been sent for publication as under :—

(*iii*) Circulatory and respiratory changes induced by electrical stimulation of the limbic system (' visceral brain '). *Jour. Neurophysiol.*, 1956 (in press).

(8) *Gastro-intestinal activity.*—The volume of grastic secretion, as well as its HCl (free and total), and pepsin contents, were both increased and decreased by stimulations of all the regions. The grastro-intestinal motility was inhibited from temporal lobe structures, and both increased and inhibited from posterior orbital and anterior cingulate.

These results have been published as under :—

(*iv*) Effect of electrical stimulation of the limbic system ('visceral brain') on gastric secretion and motility. *Ind. Jour. Med. Res.*, 1956, **44,** pp. 125-130.

(9) *Blood sugar.*—There was usually a rise in blood sugar level resulting from stimulation of all the limbic structures in the monkeys, and all these structures, except the anterior cingulate, in cat. Fall in blood sugar resulted only from stimulation of anterior cingulate in cat. (It is interesting to record here that anterior cingulate stimulation in cat produced convulsions followed by rage reaction).

These results have been published as under:—

(*v*) Blood sugar changes induced by electrical stimulation of the limbic system (' visceral brain '). *Ind. Jour. Med. Res.*, 1956, **44,** pp. 121-124.

(10) *ACTH secretion.*—Eosinophil counts in the circulating blood were also carried out both before and after stimulation of the limbic structures to study if these structures play any part in the control of ACTH secretion. The results did not show any significant results from here.

II. Surgical and electrolytic lesions in fore-brain (including ' visceral brain ').

So far 10 monkeys and 6 cats have been operated upon. The animals are studied as regards their behaviour, and their food intake and the activity of various viscera recorded for few weeks. Lesions are then made in different regions, and the post-operative behaviour, food intake, and visceral responses studied. The following lesions have been made so far.

(*a*) *Surgical lesions* :

(*i*) Bilateral frontal lobectomy (removal of both frontal lobes in front of motor area by suction).
2 monkeys and 1 cat.

(*ii*) Bilateral frontal lobotomy (cutting through both the frontal lobes by suction in front of motor area).
2 monkeys and 1 cat.

(*iii*) Bilateral temporal lobectomy (removal of both temporal lobes by suction).
1 monkey and 1 cat.

(*b*) *Electrolytic lesions* :

Electrolytic lesions have been made bilaterally in the following 'visceral brain' structures, with the help of Horsley-Clarke stereotaxic instrument.

(*i*) Amygdaloid nuclei ...	... 1 monkey and 1 cat.
(*ii*) Hippocampus ...	... 1 monkey.
(*iii*) Anterior cingulate gyrus	... 1 monkey and 1 cat.
(*iv*) Orbital surface of frontal lobe	... 2 monkeys and 1 cat.

Frontal and temporal lobectomies remove all the 'visceral brain' structures in these lobes, in addition to other structures, while the frontal lobectomy cuts their connections. Restricted electrolytic lesions would only destroy specific structures.

The results observed in these animals are summarized below:—

(1) *Food intake*.—Changes in food intake have been mainly observed after frontal lobotomy. In monkey, there has been a definite and significant increase in food intake, while the one cat with this lesion showed a slight fall in food intake. After frontal lobotomy in the monkey on the other hand, an increase in intake was observed, but was very slight. After temporal lobectomy again there was a very slight increase in food intake. After the electrolytic lesions, a very slight increase in food intake has been observed only after lesions in anterior cingulate and amygdala. Other lesions did not produce either an increase or decrease in the food intake. But after most of these lesions, the daily variations of food intake became much less.

These preliminary observations about changes in food intake after frontal lobectomy are very interesting, when one recapitulates what has been already reported by us earlier. It has been shown that in rat and cat, bilateral destruction of lateral hypothalamic regions ('Feeding Centre'), produces a complete stoppage of eating. But when such a lesion is produced in monkeys, the spontaneous eating disappears; but if the food is put into the monkey's mouth, then it swallows it. It was suggested that after higher encephalization, the higher brain centres influence the activity of the lower hypothalamic centres (feeding centres). More work is needed before the exact relationship of the higher centres can be worked out.

(2) *Circulatory changes*.—Resting heart rate has been studied in all the animals. After surgical lesions of the frontal and temporal lobes, there was generally an increase in the heart rate. After electrolytic lesions, except one monkey after amygdaloid lesions who showed a decrease in heart rate, there was no change in others. Blood pressure could be recorded only in few monkeys, by applying a small cuff on the arm and connecting it to sphygdmomanometer. One monkey after cingulate lesion showed a fall in B.P.

(3) *Respiration rate*.—A change was observed only in some animals. Monkeys after frontal lobectomy showed an increase in rate, while cats

after temporal lobectomy and amygdaloid and hippocampal lesions showed a decrease in its rate.

(4) *Blood sugar* levels have generally shown a rise after most of these lesions. The animals after temporal lobectomy showed a slight decrease in its level.

(5) *Rectal temperature.*—No change has been observed in any of the animals studied so far.

(6) *Behavioural changes* produced in these animals after these lesions were of the same general pattern, as have been already reported by various other workers. Hyperkinesis after frontal lobectomy is quite pronounced. Some definite conclusions can only be drawn after the study of more such animals.

(7) *Gastro-intestinal tract.*—One monkey after electrolytic lesions in the amygdaloid nuclei, showed marked flatulence. *X*-ray examination of the gastro-intestinal tract after Ba meal, revealed hypomotility, with balooning of certain regions.

Ulcerations have also been observed in the gastro-intestinal tract of some of those animals which have been sacrificed. Histological studies are still to be done.

The number of animals observed so far with such lesions is very few, and one cannot come to any definite conclusions on the basis of these. More studies on these lines will be carried out in the next six months.

36. Inquiry on the formation, distribution, structure and development of the splanchnic nerves under Dr. Inder Jit at the Medical College, Amritsar.

IMPORTANCE

Applied.—The value of the surgical operations in cases of essential hypertension is unquestionable, but the techniques of dealing with the condition have not been standardized as yet. A great majority of surgeons favour sympathectomy including varying degree of splanchincectomy (Allen and Adson, 1940; Peet *et al.*, 1940; Smithwick, 1940, 1944). Apart from total sympathectomy (Grimson, 1941) resulting in undesired effects and the great risk to life, these operations do not divide the splanchnic nerves higher than the lower 4 to 5 inches of their length and the lower 3 or 4 thoracic ganglia. In certain number of instances the operation fails to relieve the conditions. The cause of this failure requires investigation. Is it due to a variation in the formation and distribution of the splanchinic nerves ? At what level do the fibres arise from the splanchnic nerves to supply the aortic and œsophageal plexuses and thus provide an alternate path for the sympathetic fibres to the abdominal viscera? What should be the optimum level at which the splanchnic nerves be resected so as to interrupt the alternate pathway? Are there any *intermediate ganglia* present on the splanchnic nerves and are they thus responsible (if at all) for failure of operations ?

Fundamental.—To determine the presence or absence of the highest splanchnic nerves described by Wrisburg (1780); Ludwig (1793); Lobstein (1823); Bræucker (1927).

To find the incidence of the para-aortic nerves of Mitchell (1938).

To determine the development of splanchnic nerves.

WORK DONE.

The work could not be started earlier than 7th July, 1955 when the appointment of a Research Assistant was made. During the period ending 15th November, 1955, the following work has been completed:—

1. The origin, formation, distribution and termination of all the three splanchnic nerves have been followed on both sides in nine cadavers (18 dissections). Fine branches have been traced with the help of a dissection microscope.

2. Similar dissection has been made in one full-term fœtus.

3. The histological structure of the nerve has been examined by making serial sections in the case of one full-term fœtus. Sections were stained with appropriate techniques.

Findings.—It is not possible to give a detailed description in this brief note. As yet no conclusion can be drawn as the investigation must be extended to enable us to dissect at least fifty bodies if not hundred. The following salient points have been observed so far :—

(*a*) Variations in origin of the splanchnic nerves are of common occurrence. Uppermost root of the greater splanchnic nerve was found to arise from 5th ganglion in ten dissections and from the 6th in six dissections. It did not extend higher than 7th ganglion in two instances. In two cadavers, one on the left side and in the other on the right side, the greater splanchnic nerves arose as high as 4th ganglion by forming a loop with the 5th ganglion. Mitchell (1947) has emphatically stated that loop formation is only present between 4th and 5th ganglia. In our dissections such loop formations were seen in two instances, occurring below the 5th ganglion.

(*b*) *Lobstein's ganglion.*—It could be detected macroscopically in four dissections. In two instances in the same cadaver, it was present on the trunk of the greater splanchnic nerve before it pierced the crus of the diaphragm. In another instance where the greater splanchnic nerve divided into two branches before piercing the diaphragm, the ganglion was found on the anterior division. In the 4th instance it was present between the greater and lesser splanchnic nerves and was joined to these by means of fine twigs. These ganglia were found to give branches to the aorta and the diaphragm. In one specimen it gave a twig to the para-aortic nerve. Serial sections of a splanchnic nerve, however, showed that although these ganglia were not evident to the naked eye, microscopically four large sized ganglia and numerous small accessory ganglia could be detected.

(*c*) *Highest splanchnic nerve.*—It was described by Wrisburg (1780); Ludwig (1793); Lobstein (1823); and Bræucker (1927). Mitchell (1947) has denied its existence. He even called it a 'myth'. In the present dissections a structure resembling this nerve was found in two instances. The nerves took origin from 1st, 2nd, 3rd and 4th ganglia, from cervical cardiac nerves and from aortic and pulmonary twigs arising from 3rd, 4th, 5th and 6th sympathetic ganglia. In one dissection this nerve running along the postero-lateral aspect of the aorta entered the abdomen with the greater splanchnic nerve and ended in the cœliac plexus. In the other it ended in the greater splanchnic nerve above the diaphragm. This nerve resembled the collateral ganglionated trunk of Kondratjew (1926).

Since Mitchell (1938) affirms that para-aortic nerve never arises from above the 4th and 5th dorsal vertebræ and in one specimen this nerve co-existed with para-aortic nerve it appears that highest splanchnic nerve is a definite anatomical entity.

(*d*) *Termination of the splanchnic nerves.*—The greater splanchnic nerve after piercing the homolateral crus of the diaphragm undergoes a twist, flattens out, breaks into a leash of branches and ends in the semilunar ganglion of the cœliac plexus. Some fibres, however, go to the supra-renal gland. The lesser splanchnic nerve terminates in the aortico-renal ganglion. The least splanchnic nerve ends in the renal ganglion and posterior renal plexus. In two instances the greater and the lesser splanchnic nerves were joined by twigs of nerve fibres above the diaphragm and in other two specimens such union took place below the diaphragm.

(*e*) *Para-aortic nerve.*—The incidence of the para-aortic nerve as described by Mitchell (1938) is only 10 per cent on left side and much less on the right side. In our dissections this nerve was found in five bodies on the left side and in one body on the right side. The nerve interconnects the lowest cardiac, aortic and œsophageal branches of the sympathetic ganglia. It runs along the postero-lateral aspect of aorta, enters the abdomen through the aortic hiatus and ends in the cœliac plexus. In one instance on the right side the nerve took origin from 4th, 5th and 6th ganglia of sympathetic chain and taking a twig from uppermost root of the greater splanchnic nerve terminated in the diaphragm. In another specimen splanchnic ganglion of Lobstein gave twigs to the para-aortic nerve which on its way sent twigs to the aorta.

Results.—The present meagre work suggests that Grimson's (1941) sympathectomy is based on sound anatomical findings. If these nerves, for example, para-aortic nerve of Mitchell (1938), highest splanchnic nerve of Wrisburg (1780) and the contributions to the œsophagus and the aorta from higher ganglia of sympathetic chain are not removed as in operations suggested by Smithwick (1940, 1944) then alternate paths for sympathetic fibres to the abdominal viscera and blood vessels will remain intact. Such operations may, therefore, result in failure.

37. Inquiry on the value of commercial silk grafts to bridge large blood vessel gaps under Dr. Yudhveer Sachdeva at the Medical College, Amritsar.

This work is just a preliminary step to the problem 'The Value of Commercial Silk Grafts in bridging large blood vessel gaps.' The idea was to study methods of suturing of large blood vessels and the technique of anæthesia in dogs.

The experiments have been done on 20 dogs. The dosage of nembutal sodium as an anæthetic agent has been studied. The following methods of suturing the large blood vessels have been studied on the abdominal aorta of dogs.

1. Continuous over and over stitch end on coaptation (non-everting).
2. Continuous mattress stitch (everting).
3. Interrupted mattress stitches alternating with simple stitches (everting).

The work was started in April 1955. It was thought that before starting the use of silk grafts, it was necessary to evaluate certain suture methods of anastomosing large blood vessels, and also study nembutal sodium as an anæsthetic in dogs.

Material and methods.—The work was done on the abdominal aorta of dogs. So far 20 (twenty) experiments have been done.

Anæsthesia.—2·5 per cent solution of nembutal sodium was given intravenously. In some of the experiments ether was used in combination with nembutal.

Operation.—Abdomen was opened by a midline or left paramedian incision. Aorta was exposed and some of its branches below the renal arteries were ligated and divided so as to clear about 2″ of the aorta just above its bifurcation. Two aortic clamps were applied and aorta cut between the two. The ends were washed with saline and cleaned of adventitial tags. The ends were then sutured using Carrel's method. Suture material used was 5-0 silk on atraumatic needles. Clamps removed. Abdomen closed. No anticoagulants or antibiotics were used. Operation was done under strict aseptic precautions.

After varying periods up to a maximum of 20 days, the dogs were sacrificed, a post mortem done and condition of the anastomotic area studied.

Results.—In 15 dogs anæsthesia used was nembutal sodium alone. There was no mortality due to anæsthesia. Average initial dose used was 29·47 mg./kg. body-weight. Average total dose used was 53·60 mg./kg. body-weight. In the rest of the 5 dogs ether was combined with nembutal sodium. One of these dogs died due to anæsthesia before the completion of the operation.

In the first dog of this series aorta was clamped for half an hour and then released to see the effect of clamping. No anastomosis was done. There were no deleterious effects.

In 6 dogs the method of stitching used was continuous over and over stitch end on coaptation (non-everting)—one of these died of immediate hæmorrhage because of large size of the needles used. There was thrombosis in one. Late hæmorrhage in another. The results were good in the rest.

In the second group 6 dogs were used and the stitching was done by continuous mattress stitching (everting). In one of these there was thrombosis. One died of late hæmorrhage. Results were good in others.

In the 3rd group using alternating interrupted horizontal mattress and simple stitches, 7 dogs were used. One of these died of anæsthesia. Two of this series died of late hæmorrhage. Results were good in the rest.

38. Study of renal changes following ureteric ligation and estimation of recovery following release of obstruction by ureteric transplantation under Dr. B. N. Balkrishna Rao at the G. R. Medical College, Gwalior.

The object of the study has been to find out the critical time period for the reversal of hydronephrotic changes.

METHOD AND MATERIAL OF STUDY

(*i*) The work has been carried out in dogs. At first stage of operation in the beginning the technique of production of experimental hydronephrosis by surgical ligation of left ureter had to be standardized. This surgical ligation of left ureter is done through a left paramedian incision. Ligation of ureter has been done half inch from the urinary bladder by applying two No. 4 black silk ligatures, each being half inch from the other. After ligature the intervening portion of the ureter is excised.

TABLE I.

Shows the number of dogs in which experimental hydronephrosis has been produced.

Name of dog	Date of ligation of left ureter	Duration of hydronephrosis in days
A	22-10-54	174
B	2-11-54	51
C	11-12-54	23
D	15-11-54	52
E	11-12-54	112
F	14- 1-55	36
G	17- 2-55	175
H	2- 2-55	29
I	5- 2-55	76
K	25- 4-55	100
M	30- 5-55	109
N	8- 7-55	74
O	16- 7-55	24
P	7- 8-55	15
Q	25- 8-55	40
R	21- 9-55	Under observation.
S	30- 9-55	Under observation.
T	4-10-55	Under observation.

(*ii*) Second operation was performed in some of these dogs with a view to release the obstruction so that the hydronephrotic kidney can have a chance of reversal. For this first cutaneous uretrostomy was tried but it did not function. Uretro-colonic and uretrouretric anastomosis were tried but were abandoned as found to be not satisfactory. Now uretro-vesical anastomosis through left paramedian incision is being successfully done.

(*iii*) In the beginning of the present work to study the kidney function, entire reliance was put on the results that were contemplated to be obtained by intravenous pyelography. This procedure was tried by using various radio-opaque contrast medias, but the results were very erroneous and excretion of the contrast medias could not be standardized; however, a few good i.v. pyelograms have been obtained. Before doing i.v. pyelography dogs were prepared as in human beings. The results of the dogs submitted to this procedure are shown in Table III.

TABLE II.

Showing dogs in which the uretrovesical anastomosis has been done.

Name of dog	Duration of hydronephrosis in days	Date of uretrovesical anastomosis	Remarks
F	36	19–2–55	Dog expired half an hour after operation.
A	174	22–2–55	Dog expired on 9-3-55.
I	76	20–5–55	Right side nephrectomy done on 23-9-55.
G	175	10–8–55	Right side nephrectomy done on 29-9-55.
M	109	15–9–55	Dog expired due to over dose of anæsthetic reagent.
Q	40	3–10–55	Dog under observation.

TABLE III.

Name of dog	Date of ligation	Date of uretrovesical anastomosis	Date of intravenous pyelography	Remarks
A	22–10–54	22–2–55	5–3–55	Good pyelogram obtained.
E	11–12–54	...	12–3–55	No dye seen.
G	17– 2–55	10–8–55	12–3–55	,,
J	...	...	22–3–55	,,
L	13– 5–55	...	4–5–55	,,
N	8– 7–55	...	2–6–55	Kidney outline visualized.
O	16– 7–55	...	3–6–55	No dye seen.

(*iv*) After having abandoned the use of i.v. pyelography to study kidney function, help was sought to study kidney function by intravenous administration of one per cent 10 c.c. methylene blue and retrograde pyelography. Excretion time for one per cent 10 c.c. i.v. administered methylene blue has been standardized in normal dogs by noting the appearance and maximum intensity of blue colour through the ureteric catheter. Results are shown in Table IV.

The i.v. administration of 10 c.c. 1 per cent methylene blue had to be abandoned also due to the following reasons:—

Firstly, as is deduced from Table IV, normal time for excretion to start is 18 minutes and time for maximum intensity 23 minutes. It was thus concluded that in hydronephrosis the time excretion if at all will be so much prolonged that to keep a dog under anæsthesia is difficult and fraught with danger, specially in the light of development of a better method to study the kidney function by the use of intravenous administration of 10 c.c., 0·4 per cent of indigocarmine (*vide infra*).

TABLE IV.

Name of dog	Date of ligation	Date of administration 1 per cent 10 c.c. methylene blue	When appeared, minutes	Maximum intensity, minutes	Remarks
O	16–7–55	16–7–55	18	23	Expired on 8–8–56.
P	6–8–55	6–8–55	18	23	Expired on 22–8–55.

Secondly, it had been observed that dogs' general condition used to deteriorate after its administration and the dogs died without obvious cause.

(*v*) Due to the above drawbacks of methylene blue it was replaced by the indigocarmine 10 c.c., 0·4 per cent administered i.v. to study the kidney function and noting the appearance of blue colour in the uretric catheter (Table V) :—

TABLE V.

Name of dog	Date of ligation	Date of uretro-vesical anastomosis	Date of right side nephrectomy	Date of administration of 10 c.c., 0·4 per cent indigocarmine	Remarks
S	30–9–55	…	…	30– 9–55	Dye appeared in 2 minutes 55 seconds and maximum intensity in 3 minutes 45 seconds
R	21–9–55	…	…	21– 9–55	—do—
Q	25–8–55	3–10–55	…	3–10–55	No dye appeared from the left ureter upto 15 minutes, but appeared in bladder from right ureter.
M	30–5–55	15–9–55	…	15–9–55	,,
I	5–3–55	20–5–55	23–9–55	23–9–55	,,
G	17–2–55	10–8–55	29–9–55	29–9–55	,,

(*vi*) Retrograde pyelography is being done by the passage of uretric catheter devised in our laboratory out of the human uretric catheter. Uretric catheter is a radio-opaque one. The material used has been pyelectan retrograde. The *x*-ray factors at which the retrograde pyelograms have been obtained are as below :—

54 K.V.P.
4·5 M.A.
2·5 Seconds.

The first few times this procedure did not work well due to technical difficulty of standardization of radiographic factors and contrast media, but now it is working very satisfactorily. Results are given in Table VI :—

TABLE VI.

Name of dog	Date of ligation	Date of uretrovesical anastomosis	Date of doing retrograde pyelography	Amount of pyelectan used, c.c.	Remarks
G	17–2–55	10– 8–55	10– 8–55	10	No pyelogram obtained
M	30–5–55	15– 9–55	15– 9–55	10	Good pyelogram obtained
O	16–7–55	…	16– 7–55	4	No pyelogram obtained
P	6–8–55	…	6– 8–55	4	Good pyelogram obtained
Q	25–8–55	3–10–55	} 25– 8–55 3–10–55	4 20	„ „
R	21–9–55	…	21– 9–55	4·5	„

Retrograde pyelography and indigocarmine kidney-function tests are now being done at the same time.

(*vii*) To finally find the critical time period after having obtained a rough idea with the above procedures, it has been decided that right side transperitoneal nephrectomy in dogs be done in whom the hydronephrosis has been drained by uretrovesical anastomosis. Results are shown in Table VII :—

TABLE VII.

Name of dog	Date of ligation	Date of uretrovesical anastomosis	Date of right side nephrectomy	Remarks
I	5–3–55	20–5–55	23–9–55	Dog expired at operation due to excess of hæmorrhage.
G	17–2–55	10–8–55	29–9–55	Dog under observation.

(*viii*) Electrophoresis is being done by an apparatus devised in our department and electrophoretic pattern of serum of hydronephrotic dogs, urine and contents of the hydronephrotic sac is being studied in dogs of various time intervals.

(*ix*) Blood urea estimation is being done and the results of blood urea are shown in Table VIII :—

TABLE VIII.

Name of dog	Date of ligation	Date of uretrovesical anastomosis	Date of right side nephrectomy	Date of blood urea	Blood urea readings in mg./100 c.c. of blood
M	30–5–55	...	...	28– 5–55	23·4
				30– 6–55	24·0
				15– 7–55	23·8
Q	25–8–55	3–10–55	...	24– 8–55	23·8
				2– 9–55	25·0
				10– 9–55	24·8
P	7–8–55	...	...	6– 8–55	23·0
				11– 8–55	24·0
G	17–2–55	10–8–55	29–9–55	1– 8–55	23·8
				20– 8–55	21·6
				10– 9–55	21·8
				30– 9–55	49·0
				1–10–55	51·0
				2–10–55	57·0
				3–10–55	60·0
				4–10–55	120·0
				5–10–55	135·0
				6–10–55	145·0

(*x*) Lately, we have started subjecting the contents of the hydronephrotic sac to routine chemical examination, specially the protein contents.

(*xi*) Photographic records are being maintained at each stage of both kidneys.

(*xii*) It is proposed to take up the calorimetric estimation of the excretion of the phenolsulphonpthalein in the near future, after administration of intravenous phenolsulphonpthalein.

OBSERVATIONS AND DISCUSSIONS.

In contrast to the common textbook belief that sudden obstruction to the urinary flow leads to atrophy of the kidney, we have seen in all our dogs in which surgical ligation of left ureter has been done that invariably hydronephrosis has occurred. In not a single instance primary atrophy of the kidney following surgical ligation of left ureter has been observed. In the changes that follow the development of hydronephrosis the renal parenchyma undergoes a pressure and anæmic atrophy which advances with the persistence of the obstruction. The changes that occur in the hydronephrotic kidney have been studied under the heading of gross changes and microscopic changes.

Gross changes.—Normally, the kidney is surrounded by a small quantity of fat, mostly dispersed around the hilum and posterior surface. The perirenal fat was always found to be increased in the hydronephrotic kidney. The hydronephrotic kidneys were palpable as a reniform swelling in the left hypochondrium and left lumber region and bore all the clinical features of a reniform swelling. Pyelorenal enlargement was progressive after the surgical ligation of left ureter depending on the time for which obstruction has been kept. The normal dog's kidney varies quite widely in the dimensions as shown below :—

Length	...	5·4 to 7·2 cm.
Breadth	...	3·0 to 4·1 cm.
Thickness	...	2·4 to 3·1 cm.
Volume	...	45·77 to 78·64 cμ. cm.

The normal depth of renal parenchyma varies between 1·1 cm. and 2·4 cm. in a vertical section but with gradually increasing compression of the renal tissue by the dilating pelvis the depth decreased progressively. The variation in dog regarding dimension and surviving renal tissue following the ligation of the left ureter is shown in Table IX along with the other gross changes. Following prolonged obstruction the calyces were found to be markedly elongated and stretched from the pelvis to the periphery. This feature has been seen to be more marked in kidneys subjected to prolonged obstruction. The corticomedullary differentiation was present in kidneys subjected up to obstruction of 30 days, but later its differentiation became less distinct till at 90 days the remaining renal tissue was found uniformly homogeneous without any corticomedullary differentiation.

TABLE IX.

Name of dog	Duration of hydronephrosis, days	Dimension, cm.	Volumes, cμ. cm.	Thickness of the surviving parenchyma, cm.	Cortico-medullary differentiation	Pelvis capacity, c.c.	Remarks
B	51	8×5×3·5	140·0	0·7	Absent	35	Infection++
C	23	7×4·2×3	88·2	1·1	Present	21	Infection++
D	52	8×6×4	192·0	0·8	Absent	49	No infection
F	36	6·6×4·5×3	89·1	0·9	Very faint	30·8	,,
K	100	12×8×4	384·0	0·4	Absent	470	,,
N	74	7×4×2·2	61·6	0·6	Absent	42·8	,,
M	109	10·5×7×4	294·0	0·3	Absent	520	,,
O	24	6·1×4×2·2	53·68	1·0	Present	22·9	,,
P	15	6·1×3·4×2	41·48	1·6	Present	12	,,

Microscopic changes.—The early changes that have been observed are one of dilatation, first of the collecting tubules but later also of the convoluted tubules. The tubules after an initial dilatation rapidly exhibit degenerative changes in the epithelium and the presence of colloid material in their lumen and at a slightly later stage they disappear completely and are replaced by fibrosis. Initially, the changes following obstruction in the glomeruli are those of lobulation and hyalinization followed by fibrosis of the glomerular tuft and periglomerular fibrosis. With gradual collapse and condensation of the parenchyma the glomerulii crowded together and then gradually disappeared resisting longer than the tubules.

Uretrovesical anastomosis and hydronephrosis.—Dogs have been subjected to uretrovesical anastomosis with a view to release the obstruction. The changes in kidneys following release of obstruction have also been studied under the heading of gross changes and microscopic changes. By releasing the obstruction, a chance for the changes of hydronephrosis in kidneys for reversal is afforded and this has to be studied histologically from evidence of regeneration and by indigocarmine kidney function test.

Gross changes.—The hydronephrotic kidney after drainage by uretrovesical anastomosis shrinks invariably to a size below that of the opposite kidney. The perirenal fat which increases as a result of hydronephrosis does not alter. The depth of the renal parenchyma comes to within normal limits. The calyces which are found stretched in the hydronephrosis from the pelvis to the periphery appear thicker and shorter than the normal. The corticomedullary differentiation faintly re-appears (Table X).

TABLE X.

Gross changes after uretrovesical anastomosis.

Name of dog	Duration of hydronephrosis, days	Date of uretrovesical anastomosis	Dimension, cm.	Volume, cu. mm.	Thickness of surviving parenchyma, cm.	Corticomedullary differentiation	Pelvis capacity, c.c.	Remarks
A	174	22–2–55	5×3·5×2	35	0·5	Present	*Nil*	Drained kidney shrunk below normal size.
I	76	20–5–55	4·5×2·5×1·5	15·875	0·3	Present	*Nil*	—do—

Microscopic changes.—The kidneys that have been drained by uretrovesical anastomosis show a fairly respectable amount of renal cortex showing larger number of glomerulii, the proportion of the glomerulii in relation to the tubules is considerably more. Some of the glomerulii were showing evidence of lobulation, degeneration and atrophy of the glomerular tuft. There were areas of patchy atrophy of collecting tubules in the medullary zone and at places these areas showed replacement fibrosis with hyalinized connective tissues. The surviving tubules showed evidence of degenerative changes in the epithelial cells. The blood vessels show very marked perivascular fibrosis. There is a very little inflammatory cell infiltration. There is a good deal of condensation of connective tissue in the cortex. Since there are a fair number of tubules leading from the cortex to the pelvis, it appears that the kidney is showing some amount of functional renal parenchyma.

Value of nephrectomy in the study of the critical time period.—Recently, we have submitted a few dogs to transperitoneal nephrectomy (dog I and G). This procedure is being done in dogs in which uretrovesical anastomosis has been done. It has been observed that the dog after nephrectomy developes anuria and vomiting in a few days. Blood urea starts rising (*vide* Table XI) and dog's general condition goes down hill. Prior to nephrectomy, kidney function with indigocarmine have been tested, but in dogs G and I, which are the only animals subjected to nephrectomy at the moment did not show any evident function of left kidney. The idea to do right side transperitoneal nephrectomy is that the hydronephrotic kidney which has been drained (left, kidney) by uretrovesical anastomosis and as the right kidney that has once taken up the function may not hand over to the left kidney because of the phenomenon of renal counter balance.

TABLE XI.

Name of dog	Date of doing blood urea	Level of blood urea, mg./100 c.c.
G Nephrectomy done on 29–9–55	1–8–55	23·8
	20–8–55	21·6
	10–9–55	21·8
	30–9–55	49·0
	1–10–55	51·0
	2–10–55	57·0
	3–10–55	60·0
	4–10–55	120·0
	5–10–55	135·0
	6–10–55	145·0

Blood urea and hydronephrosis.—It has been found that the normal blood urea of dog's is between 23·5 mg./100 c.c. of blood and 27 mg./100 c.c. of blood. After the development of hydronephrosis of the left kidney no change in the blood urea has occurred, which could be explained because of the other kidney taking over function. It is proposed to do the blood urea estimation only in dogs submitted to right nephrectomy. It has been observed in dog G submitted to nephrectomy that the blood urea started rising. The maximum rise of the blood urea in dog G has been in the first 3 days after nephrectomy and was almost steady for 2 days and then again increased rapidly.

Electrophoresis and experimental hydronephrosis.—This work is still in its preliminary stage and so it is too early to predict the value of doing electrophoresis in relation to the blood serum pattern of hydronephrosis, urine and contents of the hydronephrotic sac. The matter is, however, being pursued.

It can safely be concluded from the above work and study that the critical time period is less than 5 weeks and it is hoped that this final determination of the critical time period will be obtained in the near future as it is proposed to take up the hydronephrotic dogs of duration shorter than 5 weeks. The importance of doing this work is that at what stage of hydronephrosis plastic operation be done and when should nephrectomy be done in human beings besides its experimental contribution to urology.

Below is given the brief scheme of work that is being done after having established most of the standards for finding the critical time period for reversal of the hydronephrotic changes :—

1. Production of experimental hydronephrosis of duration of 5 weeks and less.
2. Drainage of hydronephrotic kidney by uretrovesical anastomosis.
3. To do right side transperitoneal nephrectomy in dogs submitted to uretrovesical anastomosis.
4. To do indigocarmine kidney function test before anastomosis and before nephrectomy.
5. To do retrograde pyelography before anastomosis and before nephrectomy.
6. To study the blood urea after nephrectomy.
7. To do the electrophoretic pattern of serum of hydronephrotic dog, urine and hydronephrotic sac contents.
8. To do the routine chemical and microscopic examination specially protein content of the hydronephrotic sac.
9. To keep photographic record of both kidneys.
10. To submit to histopathology and to study the gross and microscopic changes comparing hydronephrotic kidney and drained hydronephrotic kidney of equal time period.

39. Inquiry into pneumoconiosis in the non-industrial population of Rajasthan with special reference to emphysema under Dr. R. K. Goyal at the Sawai Man Singh Medical College, Jaipur.

Object of the work.—It has been established that emphysema is fairly common in the non-industrial population of Rajasthan even in the younger age group. The emphysema is most probably due to some type of pneumoconiosis but no research work on this problem has been carried out to any extent in any part of the world. The chemical and microscopic examination of the soil from different parts of Rajasthan as well as the animal experiments conducted with samples of soil are bound to be of great value both from the point of view of fundamental research as well as for the general well being of the population at large.

It must be borne in mind, that it is not possible for the sand particles to maintain the exact characteristics of the rocks, from which they are derived. Considerable alterations occur and decomposition also sets in. However, the following minerals were found in the sand examined by the polarising microscope.

Jaipur dust.—It is not a typical desert sand, quartz was present in maximal amounts, the other items present sparsely were: decomposing feldspar, epidote, mica, tourmaline, and ferrugenous material. A number of quartz particles were coated with iron.

Bikaner dust.—It is a typical desert sand containing quartz to the maximal amount, small amount of decomposing feldspar, epidote and garnet. The quartz particles were coated with iron in a number of cases.

Coonoor dust.—It consisted mostly of ferrugenous clay, some quartz particles were also present.

Patiala dust.—It consisted mostly of ferrugenous material, the other items were quartz, garnet and pologonite.

Kotah dust.—It was composed mainly of ferrugenous clay, quartz was present in fairly large amounts and mica was also discernible in appreciable amounts.

Jodhpur dust.—It was not a true desert sand, the ferrugenous cement and quartz were present in appreciable amounts, feldspar was scarce and there was a small percentage of mica.

It must be borne in mind that only the desert sand can be blown about easily and thus can be noxious when inhaled, whereas the clay from non-desert regions can't be easily blown about unless there are very strong air currents and will be in general innocuous from the point of view of damage to the lungs notwithstanding the chemical composition of the soil.

It has been postulated that the dust particles of 1μ to 5μ are noxious and bigger particles are deposited only in the upper respiratory passages. The preliminary examination of the sand shows that particles less than 10μ in size are rather scanty in the soil of Jaipur. More work on the subject is in progress.

Chemical analysis.—The chemical analysis of Jaipur sand has shown the presence of 70 per cent of silicon, 0·725 g. per cent of iron, and 3·414 per cent aluminium, whereas the Bikaner sand has shown the presence of 75 per cent silicon and 2·4 per cent iron. Further work on the subject is in progress.

A number of preliminary animal experiments have been performed which show that when quartz is coated with ferric phosphate, and injected into the lungs of rabbits or guinea-pigs, a granulomatous lesion with only slight necrosis was produced. The lungs of apparently healthy adults dying of a violent death have also shown small areas of granulomatous lesions, no case of typical silicosis has been encountered so far.

40. Inquiry on toxoplasmosis—laboratory diagnosis and pilot survey under Professor H. I. Jhala at the Grant Medical College, Bombay.

The following results were obtained during the pilot survey :—

The principal tests used were:—

1. Sabin Feldman reaction.
2. Complement fixation test.
3. Toxoplasmin skin test.

TABLE I.

Results of Sabin Feldman reaction.

Group	Number	POSITIVE		Negative
		Significant titre	Insignificant titre	
Patients referred	87	10	7	70
Normal individuals	32	0	0	32
Total ...	119	10	7	92

TABLE II.

Serial number	Group	Total number investigated	Positive	Negative
1	Patients referred	140	1	139
2	Positive asymptomatic skin reactor	56	0	56
3	Pregnant women with history of abortions	80	0	80
4	Pregnant women with normal health	110	0	110
5	Kahn sera	66	0	66
6	Pyrexias of unknown origin ...	50	0	50
7	Anæmia sera	37	0	37
8	Dog sera	20	0	20
9	Ox sera	101	0	101
10	Sheep	100	0	100
11	Monkey sera	204	0	204
	Total ...	964	1	963

Comments.—Table I shows the results of the dye tests performed by us. The total tests done were 119. This included 87 patients and 32 normal individuals. Ten patients were positive in significant titres, while 7 patients showed insignificant titres. All the normal individuals showed negative results.

Table II shows the results of the complement fixation test. The total number of sera investigated is 964 which includes 525 sera from animals, such as dogs, monkeys, ox and sheep. Only one patient from a group of 140 patients showed positive test in a titre of 1 : 128. Fifty-six asymptomatic positive skin reactors were tested with negative results. One hundred and ninety sera from pregnant mothers were examined and none of them showed positive reactions. In this group of 190 pregnant mothers, 30 were with history of abortions. Sixty-six sera referred for Kahn test and 50 widal negative sera were also screened with negative results. Thirty-seven sera from anæmic cases also were investigated with negative findings.

TABLE III.

Results of Toxoplasmin skin test.

Serial number	Group	Age	Number investigated	Positive	Percentage	Negative	Percentage	U.S.A. figures positive percentage
1.	Normal adults ...	18–35	200	4	2	196	98	10
2.	Normal older persons	35–65	195	8	4·1	187	95·9	28
3.	Normal children ...	3–15	440	9	2·04	431	97·06	...
4.	Blind adults ...	15–30	300	6	2	294	98	...
5.	Blind older persons	30–50	50	2	4	48	96	...
6.	Blind children ...	3–15	50	1	2	49	98	...
7.	Pregnant mothers ...	18–35	56	2	3·57	54	98	...
8.	Children with fever, hydrocephalus, and mental retardation and convulsions	1–4	60	10	16	50	83·34	...
9.	Patients with chorio-retinitis ...	15–55	20	14	70	6	30	...
	Totals ...	...	1371	56	...	1315	...	71

Table III shows results of Toxoplasmin skin test. The incidence has varied from 2 per cent to 70 per cent. Two hundred normal adults were injected with Toxoplasmin intradermally. Four of them showed positive reactions, the positive percentage being 2. Another similar group of older normal persons was tested wherein the positive reactors were to the extent of 4 per cent. Tests on 440 normal children showed an incidence of 2·04 per cent. In an attempt to study whether the incidence was greater in totally blind population or not, we investigated blind adults (300) blind older persons (50) and blind children (50). The incidence was 2, 4 and 2 per cent, respectively. The figures are exactly the same as those obtained in healthy individuals. This leads us to state that toxoplasma parasites will not have the major rôle in complete blindness. Fifty-six pregnant mothers were tested wherein 3·57 per cent were positive. The incidence was high (60·66 per cent) in a group of children referred to us with symptoms of fever, convulsions, hydrocephalus, and mental retardation 60 such children were tested. Incidence was, however, the highest in a group of patients with chorioretinitis. Out of 20 patients tested 14 gave positive tests. Percentage was 70 per cent. Frankel (U.S.A) obtained 71 per cent incidence in a similar group. His figures for normal adults are 10 per cent, while for normal older persons are 28 per cent. Figures obtained here are too low as compared with those obtained by Frankel (U.S.A) in normal individuals.

The following are the brief notes of cases diagnosed by us as cases of toxoplasmosis :—

Case I.—Patient K. G., aged 35, complained of gradual loss of vision. The right eye showed central macular choroiditis. The margin was irregular and pigment proliferation was observed in the centre of the path. *X*-ray of skull showed cerebral calcifications. The toxoplasmin test was positive. The dye test was positive in a titre of 1 : 64, while the complement fixation test was negative. No repeat testing was possible.

Case II.—Patient A. S., aged 55 had no clear vision. Fundus showed bilateral central chorioretinitis. The patches were circumscribed with clear-cut margins and pigment deposits in the centre. The skin test was positive. The dye test was positive in a titre of 1:64. On repeated testing after 2 months the titre remained at the same level. The complement fixation test was negative.

Case III.—Patient H. R., aged 22 gave history of strong fever with cutaneous eruptions at the age of 4 years. The patient developed convergent squint in both eyes and vision deteriorated. Fundoscopy showed patches of chorioretinitis. The skin test was strongly positive. The dye test was positive in a titre greater than 1 : 64. The complement fixation test was negative.

Case IV.—Patient N. M., aged 55, had retinitis proliferance. He had a mature cataract in right eye and was operated. After operation he developed severe iritis. Toxoplasmin skin test was strongly positive. The dye test was positive in a titre of 1:1024 which on repeated testing for six months remained constant. The complement fixation test was negative.

Case V.—Patient R. B., aged 2½ years, had convulsions and fever at the age of 2 months. The child also showed mental retardation. *X*-ray of skull showed mild hydrocephalus. The fundus was normal. The optic discs were pale. The skin test was weakly positive. The dye test was positive in a titre of 1:16, which within a month reached a titre of 1:128. The complement fixation test was negative.

Case VI.—Patient H. R. M., aged 2 years, was a premature baby and was congenitally deaf and dumb. She had fever for 8 months. She showed mental retardation. The skin test was positive and so was the dye test in a titre of 1:16 while the complement fixation test was negative.

Case VII.—Patient A. H. had normal vision till 19 years of age. He had an attack of small-pox which was later followed by heavy fever diagnosed by local doctors to be due to typhoid. The patient's vision started deteriorating then. On fundoscopy both eyes showed chorioditis. He had a close contact with goats and dogs. The vision could not be corrected with glasses. Hence, it was suspected to be due to toxoplasmic infection. The Sabin Feldman dye test was positive in a titre of 1:512 which went down to 1:256. The C.F. test was negative. The *x*-ray of the skull had shown maxillary sinusitis. No calcifications were detected.

Case VIII.—Patient A. Sh., aged 17 years, gave the history of fever, diarrhœa and vomiting till 9 years of age. He had congenital defective vision which could not be judged before he was admitted to the school. The fundoscopy revealed chorioretinitis in both eyes. The skin test was positive. The dye test was positive in a titre of 1 : 64. The C.F. test was negative.

Case IX.—Patient P.V., aged 10 years, had occasional fits and mental retardation. There is history of abortions in the family. Toxoplasmin test was positive, and dye test was positive in 1:64. Mother's toxoplasmin test was also positive. Her dye test was positive in 1:16. C.F. test was negative in both. The laboratory findings suggest a possibility of the transplacental transmission to the child *in utero*. Probably the mother is a carrier.

Case X.—Patient R.M.K., aged 1½ years, had fever at the age of one year. He has hydrocephalus and mental retardation. The skin test was negative but the dye test was positive in 1:16. The C.F. test was negative.

Case XI.—Patient S., aged 7 years, had a patch of chorioretinitis in the macular in the left eye. She had fever at the age of 4 years. *X*-ray of the skull showed calcifications over left parietal region. The complement fixation test was positive in a titre of 1:128, while the skin and the dye tests were not done.

EXPERIMENTS.

(1) An attempt to isolate the parasite from the aborted products of of pregnant mothers was also made. The aborted products of different ages were collected. A suspension was made in saline. Appropriate amount of penicillin and streptomycin was added and the suspension was inoculated intraperitoneally in mice. A blind passage was made on the fifteenth day. These mice were observed for one month. Out of 50 isolations attempted by us none of them gave positive findings. No toxoplasma could be isolated.

(2) *Study of morphological behaviour of toxoplasma.*—Toxpolasma gondii is usually regarded as a non-motile parasite. On every passage a cover-slip preparation was made sometimes on the 3rd day after inoculation. Occasionally, it was observed that some of the toxoplasms were showing movement. They changed their position by moving in one direction. The movement looked like flagellar one. Rest of the toxoplasms in the field, however, remained non-motile. The number of the motile toxoplasms was also very small. This has also been observed by Cross, who mentions that 50 flagellated toxoplasma were observed in 3 slides. It still remains to be decided, whether the life cycle involves the motile phase of the parasite.

(3) *Transmission of toxoplasma by male mouse to a female mouse during coitus.*—The male prepuce was infected with toxoplasma parasites and the mice were caged with normal adult female mice. The female got pregnant and this was observed on 10th day as the abdomen was enlarged. The young ones that were at full term were quite healthy. The male and

the female also did not die of toxoplasmosis. This does not enable us to conclude anything as it may be just possible that the male did not take the infection or transmission was not possible by this route. Cowen infected prepuce of adult mice and caged them with female mice but he could not transmit the infection.

(4) *Presteimerin sensitivity test.*—A recently isolated antibiotic, presteimerin was tried. This experiment has been reported in our previous publication as ineffective in preventing disease or prolonging survival in experimental infection in mice.

(5) *Preparation and supply of Toxoplasmin.*—We have been preparing toxoplasmin skin test antigen from chorioallantoic membranes having a rich growth of toxoplasma. The method is that of Frankel and is described in detail in our earlier publication. We have sent toxoplasmin and its control to all medical institutions.

DISCUSSION.

Beattie and Bevarley have standardized Sabin-Feldman reaction. They state that the following titres are significant :

Age	Titre
Under ten years ...	... 1:16 or over.
Between ten and nineteen years	... 1:32 or over.
Over twenty years ...	... 1:64 or over.

Taking this as a suggestive titre, we may say that 10 cases out of the 17 cases cited in Table 1 show an evidence of toxoplasmosis. The skin test was positive in all these. The complement fixation test was negative. The American Committee on Toxoplasmosis, however, suggests that on repeated testing the dye test titre should show an increase of eight-fold and should be at a level of 1:256 for several weeks. It had been possible for us to do repeated testing on cases 5, 6 and 7. The results are as follows:

Name	Date	Titre
N.M. ...	2-3-55 ...	1 : 1,024
	2-4-55 ...	1 : 1,024
	2-6-55 ...	1 : 1,024
R.B. ...	22-2-55 ...	1 : 16
	14-3-55 ...	1 : 128
A.H. ...	2-3-55 ...	1 : 512
	3-4-55 ...	1 : 256

Thus, it is concluded that these three cases are definite cases of toxoplasmosis. The C.F.T. in all the three has remained negative. Besides the above three cases, case (S) whose C.F. test was positive in 1 : 128 is also a proved case of toxoplasmosis reported by us.

The transmission of toxoplasmosis is yet unknown. It is, however, suspected that there are animal reservoirs, and the infection was transmitted by the domestic animals. A recent survey in dogs has shown a very high incidence of toxoplasmosis. This is reported by Steele. We have reported the incidence of toxoplasmin reactors as 2 per cent in young adults and four per cent in normal older persons. These figures are comparatively very low as Frankel has reported 28 per cent sensitivity in a similar group in U.S.A. It is not customary in India to have animal pets in the houses and hence such a low incidence can be justified. We obtained a small quantity of toxoplasmin from U.S.A. and Vienna. Both of these have given the same results as the ones obtained by toxoplasmin prepared by us. Our figures for the group of patients having chorioretinitis are in fair agreement with those of Frankel.

Regarding the life cycle, most of it is unknown. Cross has, however, suggested the life cycle as follows: He is of the view that the pseudocyst is not a cyst but it is a 'terminal' stage in leucocytic parasitization. The leucoytes are invaded either by elongated or spherical parasites wherein the parasites multiply to the maximum capacity and the cell wall bursts to free the organisms, or that the cell wall may act as a resistant wall enclosing the parasites. Frankel has also suggested that the parasites are freed as a result of the rupturing of pseudocyst. Until a definite key is available to us it is very difficult to guess the life cycle of toxoplasma gondii.

SUMMARY.

As evidenced by remarkably low toxoplasmin rate, toxoplasmosis is a rare infection in Bombay as compared to most of the Western countries, including U.S.A. The fact that we have come across some positive skin reactors and eleven cases, shows that toxoplasmosis is occurring in Bombay. In symptomatic selected group of patients, the toxoplasmin reactivity rate reported is as high as is in the U.S.A. This shows that while the diseases of toxoplasmic origin are existing in this country, the healthy individuals show a comparatively very low incidence, due to perhaps lesser chances of getting infected with toxoplasma. This is further supported by the negative serological tests in limited number of seras from 4 species of local animals. It is likely that intimate contact with infected pets would predispose to increased reactivity to toxoplasmin in West but such chances are few and far between in India. Figures of reactivity to toxoplasmin in other parts of India are awaited with interest.

The motility in toxoplasma has been reported.

Sexual mode of transmission of toxoplasmosis in experimental mice was studied with negative results.

We have supplied toxoplasmin skin test antigen to all the medical colleges in India.

41. Investigation in the use of certain Ayurvedic preparations in the treatment of *Diabetes mellitus* under Dr. R. V. Sathe and co-workers at the J. J. Hospital & Grant Medical College, Bombay.

Work done previously.—Dr. S. S. Ajgaonkar, one of the co-workers, has already worked on the problem and has used zinc (Jasad) bhasma in combination with other preparations with successful results in cases of diabetes of maturity onset type, where no irreversible changes have taken place. This work has appeared in two papers*. These minerals have been found to be very useful in *Diabetes mellitus* of the type referred to above. In these cases, after treatment with Ayurvedic minerals in combination, blood sugar comes to normal level and there is no sugar in 24-hour urine without insulin. Cases with some irreversible changes already receiving insulin do react satisfactorily, but not to the same extent. These minerals reduce the requirements of insulin by about 40 units. In general, the patients feel better, their blood pressure is reduced (if raised) in many cases. They put on weight and the signs and symptoms of diabetes are ameliorated appreciably.

During the period of 3½ months seven cases of *Diabetes mellitus* were investigated. Six of these were males and one female. Five of them were Hindus, one Muslim and one Christian. On an average their ages varied from 41 to 55 years. Thus, the patients, as a class, belong to the maturity onset type of diabetes. Their heights varied from 5′–1½″ to 5′–8″. The difference between the previous maximum weight (before the onset of syndrome) and the present weight (on admission) of the patients varied rom 7 lb. to 31 lbs.

SELECTION OF CASES.

Once in a week the patients attending the Diabetic Clinic of the Out-patients Department of Sir J. J. Group of Hospitals are examined for their urine sugar excretion. Important cases out of these are investigated for the glucose tolerance curve, blood urea, and blood cholesterol. So far, 30 such cases have been studied. On this basis, cases are selected for the stay in hospital for treatment and a day to day follow up. This, of course, depends upon the co-operation of the patients. The period of stay of the patients varied from 1 month (minimum essential for study) or more depending upon the severity of syndrome and the individual response to the treatment.

CASE AND CLINICAL HISTORY.

All the seven patients complained of thirst, polyurea, headache, loss of weight, weakness, pain in legs and difficulty in healing of wounds. Two patients (case Nos. 2 and 4) had retinal hæmorrhages and disturbed vision and one (case No. 7) had fistula in ano.

From their data of family history, it was revealed that three of them had at least two members of their family suffering from *Diabetes mellitus*.

DIETARY BACKGROUND.

All the patients were non-vegetarians. As they knew they were diabetics, they avoided sweets, sugars and rice but took chappati, bread, legumen, vegetables and little milk *plus* non-vegetarian food.

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*Ajgaonkar, S. S.—1. "The role of Ayurvedic Medicines in the treatment of Diabetes Mellitus"—Indian Journal of Medical Sciences—Vol. 6, No. 2, Feb. 1952, pp. 117-136.

2. "Review of Diabetic cases treated with Ayurvedic Medicine" "Special Therapeutic Number" of the Indian Medical Review—1954.

RESULTS.

(*a*) *Urine volume.*—The 24-hour urine excretion of all the patients on admission was found to be 2 to 2·5 litres. During treatment, this figure came down to 1 litre or little below it. This, however, is a passing phase and finally it soon reaches the normal limits—1·2 to 1·5 litres.

(*b*) *Urine sugar and blood sugar.*—The fasting urine sugar on admission varied from 0·4 g. per cent to 4·5 g. per cent. This on discharge was found to be *nil* in all except in one which showed traces of sugar.

The fasting blood sugar of all the cases was considerably high on admission. It varied from 173 to 324 mg. per cent. Except in case of one patient (case No. 3), all the patients had their fasting blood sugar values raised above 190 mg. per cent. At the time of discharge, these blood sugar values were considerably lowered. This figure varied from 115 mg. to 208 mg. per cent. Except in one case (case No. 2), all the blood sugar levels were below 160 mg. per cent.

It is interesting to note that high blood sugar levels observed on admission do not go parallel with those of urine sugar excretion observed simultaneously. Case No. 2 had fasting blood sugar value of 324 mg. per cent with 1·8 g. per cent urine sugar excretion, whereas case No. 7 which had 212 mg. per cent blood sugar had 4·5 g. per cent fasting urine sugar.

The blood sugar levels in three cases (Nos. 2, 3, and 4) did not show marked alteration as a result of the treatment as compared with other cases. This may be due to the complications of the syndrome observed in case Nos. 2 and 4 and the evidence of liver dysfunction in case No. 3—*see* (d) and (f).

(*c*) *Blood urea and blood cholesterol.*—These were found to be within normal range throughout the period of stay. The blood urea varied from 22 to 38 mg. per cent, whereas blood cholesterol varied from 115 to 188 mg. per cent. At no stage, any deviations comparable with sugar levels occurred in either. Moreover, there was not found any correlation between the blood urea and blood cholesterol values. Our results entirely agree with those of Bodansky (Biochemistry of Diseases—Bodansky) who found no relation of these values with the diabetic syndrome.

(*d*) *Liver function tests.*—Icterus Index was found to be normal in all cases, which varied from 1 to 7 units. Van den Burgh direct was negative in all cases. This shows that there was no lesion of the billiary ducts.

Thymol turbidity test was higher than the normal range of 1 to 5 units except in one case. In the rest, this varied from 7 to 12 units. Cephalin cholesterol test showed no liver dysfunction except in two cases (Nos. 2 and 3) where it gave doubtful 1 *plus* reading.

(*e*) *Urea clearance test.*—Urea clearance test varied from 52 to 100 per cent of normal average clearance. This also remained normal throughout the treatment. It won't be irrelevant, at this stage to state that urea clearance test was also performed on four normal healthy adults (22 to 25 years), who gave the results from 61 to 68 per cent of normal average clearance. These values, slightly below normal per cent (70 per cent) may be due to the climatic condition and the food habits in India. This observation agrees with the recent publication (*Jour. Post-Gr. Med.*, 1955, p. 79, Chitre *et al.*).

TABLE I.

Case number	Observations	Blood sugar curve, mgms. per cent					Urine sugar curve gms. per cent					Blood urea, mg. per cent	Blood cholesterol, mg. per cent	Liver Function Tests					Urea clearance test NACl per cent	Serum proteins		
		F	1	2	3	4	F	1	2	3	4			Liver icterus index units	Vanden Burgh direct	Thymol turbidity units	C.C.F. Tests 24 hours	C.C.F. Tests 48 hours		Total proteins G. per cent	Albumins G. per cent	Globulins G. per cent
1	On admission	280	320	340	280	248	1·8	2·0	2·5	2·5	2·8	30	156	1	—ve	9	—ve	+	57·5	7·6	4·0	3·6
	On discharge	119	...	334	...	...	*Nil*	...	3·7	...	...	32	171	5	—ve	11	—ve	—ve	68·0	7·6	4·4	3·2
2	On admission	324	360	400	400	380	1·8	2·0	2·8	3·3	2·8	23	156	4	—ve	5	+	+	70·0	7·8	4·9	2·9
	On discharge	208	...	264	...	...	Tr.	...	0·5	...	...	26	149	3	—ve	4	+	+	70·5	7·5	4·1	3·4
3	On admission	173	219	255	226	200	0·5	0·6	1·0	1·5	2·0	22	115	3	—ve	12	+	+	100·0	7·7	3·7	3·9
	On discharge	159	...	272	...	...	*Nil*	...	2·5	...	...	38	130	3	—ve	10	+	+	70·2	7·6	3·9	3·7
4	On admission	188	282	291	260	224	0·4	1·2	2·5	2·8	2·0	23	144	4	—ve	8	—ve	—ve	60·0	7·4	4·8	2·6
	On discharge	163	...	189	...	...	*Nil*	...	Tr.	...	...	24	160	5	—ve	8	—ve	—ve	62·0	7·4	4·7	2·7
5	On admission	224	327	340	358	358	4·0	4·0	4·0	4·1	4·3	30	188	4	—ve	7	—ve	—ve	52·0	6·2	4·1	2·1
	On discharge	140	...	220	...	...	*Nil*	...	Tr.	...	...	27	170	2	—ve	7	—ve	—ve	57·8	6·7	4·3	2·4
6	On admission	195	217	260	283	260	Tr.	0·4	1·1	2·0	2·3	30	170	7	—ve	9	...	...	62·0	6·1	4·0	2·1
	On discharge	135	...	...	...	...	*Nil*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
7	On admission	212	243	308	273	265	4·5	5·2	6·1	7·0	7·0	29	177	4	—ve	8	—ve	+	88·5	6·8	4·1	2·7
	On discharge	115	...	199	...	...	*Nil*	...	Tr.	...	...	29	170	3	—ve	7	+	+	...	7·2	4·8	2·4

TABLE II.

Case number	Period, days	Diet*	Insulin Quick and Long acting units		Jasad Bhasma, grains	Blood sugar fast, mg. per cent	Urine sugar fast, g. per cent	24-hours urine sugar, g.	Weight, lb.	Blood pressure and remarks.
1	1st	B.F.D.	*Nil*	*Nil*	*Nil*	280	...	82·3	124	115/70
	5th	D.D.	,,	,,	,,	...	...	130·7	...	...
	10th	,,	20	20	,,	...	...	22·5	...	...
	23rd	,,	24	24	,,	...	...	59·4	126	...
	30th	,,	28	28	,,	...	...	42·3	...	...
	37th	,,	32	32	2TDS	...	...	35·9	127	...
	44th	,,	28	32	,,	...	...	10·0	...	...
	52nd	,,	28	28	,,	...	...	5·8	...	...
	56th	,,	24	24	,,	156·3	*Nil*	Trace	...	...
	63rd	,,	20	20	,,	130·4	,,	22·0	...	...
	67th	,,	16	20	,,	...	...	11·7	...	...
	83rd	,,	16	20	,,	119·0	*Nil*	16·7	130	Discharged.
	86th	H.D.	*Nil*	*Nil*	,,	...	...	8·5	...	...
	92nd	,,	,,	,,	,,	...	...	*Nil*	...	...
	104th	,,	,,	,,	,,	...	...	17·0	...	(Attack of influenza).
	113th	,,	,,	,,	,,	...	...	6·7	...	...
	120th	,,	,,	,,	,,	...	...	9·4	...	...
2	1st	B.F.D.	*Nil*	*Nil*	*Nil*	324·0	1·8	25·0	184	170/100
	10th	D.D.	,,	,,	,,	...	...	57·0	...	...
	14th	,,	20	16	,,	...	...	32·0	...	...
	21st	,,	20	16	,,	...	...	5·0	...	...
	28th	,,	20	16	,,	...	...	*Nil*	...	...
	29th	,,	*Nil*	*Nil*	2TDS	289·0	...	Trace	...	...

TABLE II.—(*Contd.*)

Case number	Period, days	Diet*	Insulin Quick and Long acting units		Jasad Bhasma, grains	Blood sugar fast, mg. per cent	Urine sugar fast, g. per cent	24-hour urine sugar, g.	Weight, lb.	Blood pressure and remarks.
2	35th	D.D.	*Nil*	*Nil*	2TDS	...	...	14·0	...	160/100
	44th	,,	,,	,,	,,	...	...	Trace	...	...
	48th	,,	,,	,,	,,	238·0	...	*Nil*	...	135/90
	54th	,,	,,	,,	,,	...	...	Trace	...	...
	59th	,,	,,	,,	,,	198·0	...	11·7	...	Infection.
	68th	,,	,,	,,	,,	208·0	...	9·0	180	150/90
3	1st	B.F.D.	*Nil*	*Nil*	*Nil*	173·9	...	49·0	121	Discharged 140/90
	3rd	D.D.	,,	,,	,,	...	...	47·0	122	...
	6th	,,	,,	,,	2TDS	...	...	14·0	...	...
	12th	,,	,,	,,	,,	165·2	...	9·9	123	...
	24th	,,	,,	,,	,,	...	...	6·5	...	...
	38th	,,	,,	,,	*Nil*	159·0	...	10·0	...	(Left against medical advice)
	43rd	H.D.	,,	,,	2TDS	...	...	*Nil*	...	...
	87th	,,	,,	,,	,,	...	...	,,	...	...
	98th	,,	,,	,,	,,	125·0	*Nil*	,,	...	...
4	1st	B.F.D.	*Nil*	*Nil*	*Nil*	187·8	0·4	16·0	149	170/90
	11th	D.D.	,,	20	,,	...	...	13·7	...	...
	19th	,,	12	12	,,	...	...	9·2	...	...
	26th	,,	*Nil*	*Nil*	2TDS	176·6	...	20·0	...	...
	31st	,,	,,	,,	,,	...	...	9·0	149	150/85
	37th	,,	,,	,,	,,	...	...	Trace	...	...
	43rd	,,	,,	,,	,,	...	...	*Nil*	...	...

TABLE II.—(*Concld.*)

Case number	Period, days	Diet*	Insulin Quick and Long acting units		Jasad Bhasma, grains	Blood sugar fast, mg. per cent	Urine sugar fast, g. per cent	24-hour urine sugar, g.	Weight, lb.	Blood pressure and remarks.
4	50th	D.D.	*Nil*	*Nil*	2TDS	163·0	...	*Nil*	...	...
	58th	,,	,,	,,	,,	...	...	,,	...	Discharged.
5	1st	B.F.D.	*Nil*	*Nil*	*Nil*	224·0	4·0	62·0	118	120/70
	4th	D.D.	20	20	,,	...	...	58·0	...	...
	11th	,,	28	24	,,	...	...	43·7	...	...
	17th	,,	28	24	,,	...	...	*Nil*	120	...
	21st	,,	*Nil*	*Nil*	2TDS	...	...	17·6	...	...
	24th	,,	,,	,,	,,	188·0	...	7·9	...	...
	30th	,,	,,	,,	,,	...	...	Trace	...	...
	38th	,,	,,	,,	,,	...	...	*Nil*	...	...
	44th	,,	,,	,,	,,	135·0	...	,,	123	110/70 Discharged.
7	1st	B.F.D.	*Nil*	*Nil*	*Nil*	212·0	4·54	38·0	128	140/90
	7th	D.D.	,,	,,	,,	...	...	55·0	...	...
	8th	,,	20	20	,,	...	...	56·0	...	...
	13th	,,	16	20	,,	...	...	4·1	...	...
	15th	,,	16	20	,,	...	...	*Nil*	...	...
	19th	,,	*Nil*	*Nil*	2TDS	126·0	...	4·3	...	...
	21st	,,	,,	,,	,,	...	...	Trace	...	...
	23rd	,,	,,	,,	,,	...	...	*Nil*	...	...

*B.F.D. = Basal full diet (2,400 calories and 355 g. carbohydrates).
D.D. = Diabetic diet with—(1,760 calories and 200 g. carbohydrates).
H.D. = Home diet, medically prescribed but more liberal.

(*f*) *Serum proteins.*—The total serum proteins in all the cases on admission and on discharge were found to be normal varying from 6·1 to 7·8 g. per cent. The albumin/globulin ratio was also normal except in case No. 3. Incidentally, this case also gave the thymol turbidity raised to 12 units and the cephalin cholesterol flocculation test doubtful 1+.

(*g*) *Hæmatological studies.*—The r.b.c. count in all cases varied from 4·1 to 5·6 mill. cmm., w.b.c. from 5,600 to 9,500 cmm. (except in case No. 7 who had fistula in ano gave a figure of 18,600), hæmoglobin from 11 to 17·6 g. per cent and the E.S.R. from 4 to 8 mm. (except in case No. 4 who showed Kahn test positive in 1:8 dilution gave E.S.R. 30 mm.).

(*h*) *Body-weight and blood pressure.*–The average increase in weight of the patients during their stay in the hospital was found to be 3 to 4 lb. in spite of the fact that they were on a diet considerably low in calories—1,760. This proves that only the proper utilization of the available carbohydrates helped.

Case No. 2, who was obese, had decreased in weight by 4 lbs. Case No. 4 did not show any change probably because he was ill for about 10 days due to infection.

Blood pressure was reduced as a result of the treatment in two cases (2 and 4), which were hypertensive previously. In the rest, it was normal throughout.

(*i*) *The 'Jasad Bhasma' treatment.*—After keeping the patient on diabetic diet (1,760 calories and 200 g. carbohydrates) for 3 to 4 days, a minimum dose of 20 units of insulin was started which was increased or decreased according to individual requirement as shown by the daily urine sugar examination.

Usually (case Nos. 2, 4, 5 and 7) after ascertaining the requirement of insulin to make the patient aglycosuric, 'Jasad Bhasma' tablets (6 grains per day) were started and the effects studied—omitting insulin. It is observed that at this stage the urine sugar excretion is again found to rise a little because of the withdrawal of the immediate support of insulin. This, however, is a passing phase and soon the patients gradually become aglycosuric with 'Jasad' tablets alone. The urine volume which was high previously also becomes normal at this stage.

In case No. 1, the patient was administered 40 units of insulin in the beginning, when he was excreting 130 g. of sugar in 24 hours with 3,900 c.c. of urine volume. In 20 days this dose of insulin had to be increased by 24 units (total 64 units) when the patient excreted 35 g. of sugar per day with 1,850 c.c. urinary volume. As the patient did not become aglycosuric even after treatment with insulin for 4 weeks, the 'Jasad bhasma' treatment was also started simultaneously with insulin. Due to this insulin requirement was reduced from 64 to 56 units in 15 days and then to 32 units in 26 days. The patient was discharged on 76th day and was kept on zinc tablets only, without insulin. Thirteen days after his discharge, he showed no sugar in urine with 850 c.c. of urine volume on home diet. Thus, 'Jasad Bhasma' successfully replaced insulin administered in the beginning as control treatment. Very recently, he had an attack of influenza and thereafter he relapsed and passed 9·4 g. of sugar per day. It will be noted that he was passing 130 g. of sugar per day when admitted.

Only one patient (case No. 3) was kept on the 'Jasad' tablets alone from the beginning *without insulin.* On basal diet he passed 49 g. of sugar in 24 hours. He was kept on diabetic diet when he passed 47 g. of sugar per day. The zinc tablets were started when he excreted 14 g. of sugar in 24-hour urine, for few days. The patient became aglycosuric 38 days after the 'Jasad Bhasma' treatment was started; with 159 mg. per cent blood sugar. Two months after his discharge, his blood sugar was found to be 125 mg. per cent, on home diet and with 'Jasad' tablets only.

Case No. 6 who was discharged within 12 days from the hospital is not represented in Table II. However, this case is interesting so far as he was kept *only on diabetic diet* without either insulin or zinc tablets. With basal diet, he was passing 7 g. of sugar per day. On diabetic diet, he passed traces of sugar for 3 days and there was no sugar detected afterwards till he was discharged. Obviously, he had a very mild type of diabetes, although his blood sugar was 195 mg. per cent on admission which came down to 135 mg. per cent on discharge.

Four patients (case Nos. 1, 3, 6 and 7) who reside locally in Bombay, are attending the diabetic clinic of Sir J. J. Group of Hospitals, weekly for check-up and advice. They have been so far found to be aglycosuric on controlled but more liberal home diet with necessary 'Jasad' treatment.

42. Inquiry on the incidence of atherosclerosis in Delhi State and its correlation with blood under Dr. S. Padmavati at the Lady Hardinge Medical College, New Delhi.

Clinical material used in inquiry

Three types of cases have been studied so far.

1. The urban population of Delhi as exemplified by the hospital class of patient. This includes manual workers, professional men, clerical workers etc. and it has not been possible to sort them out on an occupational basis. Patients from the Irwin, Safdarjung and Lady Hardinge Hospitals have been included in this study.

The general practitioners of Delhi were circularised through the Delhi Medical Association and have co-operated in this study by referring suitable cases for the research programme.

2. Industrial workers from the Delhi Cloth Mills are being studied. This aspect of the inquiry could not be started until late owing to lack of a Research Fellow (already mentioned). The mills employ some 10,000 workers and the present plan is to study 1,000 of them below the age of 45 over a number of years.

3. The rural population of Najafgarh is also on the programme. Work here has just begun. The main difficulty was in obtaining transport (the Primary Health Centre at Najafgarh is some 20 miles from Lady Hardinge Hospital) and lack of a doctor to do the work. These difficulties are gradually being overcome and by early next years it is hoped to have regular by-weekly clinics at Najafgarh.

Note.—Clerical workers who come under the Contributory Health Scheme of the Central Government will form a separate category for study next year.

So will private patients (pooled from several sources) without whom a satisfactory picture of the disease will not be possible.

METHOD OF INVESTIGATION.

1. Hospital patients are studied at the Cardiac Clinic. A special proforma No. 1 (attached) is used in the interrogation and examination of patients. A through deitetic history is taken. An attempt has been made in this inquiry to correlate, as far as possible the incidence of atherosclerosis with dietary fat, community-wise, whenever convenient.

In addition a special group of patients are being intensively studied. These are "young atherosclerotics" below the age of 40 years. In this class of patients, full biochemical studies are being made such as blood chlesterol (total, free and ester) phospholipids and blood uric acid, in addition to the usual through clinical check-up (which includes Somatotyping, EKG and X-ray studies). We have at the present time ten patients in this group. For the present, the determination of cholesterol, is done by the rapid method of Zak, Dickerman, White, Burnett and Cherry. (*Am. J. Clinical Pathology*, **24,** 1307, 1954) with a Leitz-Rouy Photrometer. For determination of Phospholopids, the method of Zilversmit and Davis is being used. These techniques may have to be changed for improved methods in the future.

We also hope to study, early next year, lipo-protein molecules in atherosclerosis, with the aid of the ultracentrifuge at the University of Delhi, Physics Department.

2. Industrial workers at the Delhi Cloth Mills are interrogated and examined on a separate proforma. It is proposed to study one tenth of the total number of workers (in order to make the inquiry statistically significant) over a period of several years in order to see the effect, if any, of occupation. A series of blood cholesterol and phospholipid studies are being made on a number of these workers to determine the normal levels in the population.

3. The Rural Population is also being studied with the use of the special proforma in order to determine the incidence among the rural population. Some cases from this group have been moved into group (I) for special study.

It must be emphasized that patients in Groups II and III are normal cases, in whom an attempt is made in the course of the enquiry, to study the incidence of atherosclerosis.

Ideally, all patients in groups two and three should have, in addition to the routine physical check-up EKGs and X-rays. Owing to lack of funds and staff, it has been possible to do this only in about 20 per cent of them.

RESULTS OBTAINED SO FAR.

It is not possible to give a satisfactory report during this short period (April to September, 1955). This is the first enquiry of its kind being done in India and many difficulties, quite unexpected, had to be surmounted in getting the work organized. It is hoped that next year the work would go smoothly.

(i) *Incidence of atherosclerosis:*

No proper figures exist at the present time regarding the relative incidence of atherosclerosis among the population of Delhi or for that matter of most places in India. Hospital figures from the Out-patients departments are extremely unreliable owing to the overcrowding and consequent hasty diagnoses made. Figures taken from ward admissions are more reliable (although not absolutely so) and those given below are for a four year period (1950-54) from the Irwin, Safdarjung and Lady Hradinge Hospitals. Date previous to 1951 were considered too unreliable to merit study.

The total number of cases labelled atherosclerosis during this period in all three hospitals was 173. The number of cases at the Lady Hardinge Hospital was 21, (all women) forming five per cent of all types of heart disease treated and 0·29 per cent of all admissions. At the Irwin Hospital they numbered 107, forming nine per cent of all types of heart disease and 0·18 per cent of all admissions in the hospital. At the Safdarjung Hospital, there were 45 cases during this period, forming 1·2 per cent of all admissions in the hospital.

The age incidence was as follows:

Men		Women	
10–20 years	Nil	10–20 years	2·14%
20–30 ,,	3·24%	20–30 ,,	8·69%
30–40 ,,	11·82%	30–40 ,,	5·83%
40–50 ,,	24·73%	40–50 ,,	34·48%
50–60 ,,	40·86%	50–60 ,,	34·48%
Over 60 years	19·35%	Over 60 years	14·38%

Allowing for inaccuracies in diagnosis and poor statistics generally, the figures would indicate that atherosclerotic heart disease forms eight per cent of all types of heart disease in Delhi. It is interesting to compare this figure with those in other countries e.g. Ceylon—2½ per cent among hospital patients and (ten per cent among private patients). England, ten per cent among hospital patients (41 per cent among private patients), U.S.A. (New York City) 18·4 per cent (Virginia) 46 per cent (OHIO) 22·4 per cent and so on.

(ii) *Dietary fat:*

The estimation of fat in the diet was done by interrogation as per proforma mentioned. The amount of fat in the diet was then claculated from tables for India. It was possible by this method to estimate roughly the amount of animal fat ingested but not of vegetable fat. Actual weighing of the diets to determine separately the vegetable and animal fat in the diet is not possible at this juncture.

The amount of animal fat consumed per day ranged from 89 gms. to nil with an average of 26 gms. per day. It is interesting to note in this connection that the daily dietary intake in the " young " atherosclerotic group was considerably higher, being on an average 52 gms. per day, although this is nowhere near the daily consumption of fat per head in the U.S.A.

(iii) *Cholesterol and Phospholipid estimations:*

The average levels of Serum cholesterol (free, total and easter) and phospholipids and the cholesterol/phospholipid ratio were as follows in 75 cases):—

	Range	Average	Normal values in other countries (U.S.A.)
Total cholesterol	115-290 mg.	174 mg.	120-260 mg.
Free cholesterol ...	30-145 mg.	60·5 mg.	72-220 mg.
Cholesterol Ester ...	85-145 mg.	113·5 mg.	80-180 mg.
Phospho-lipids (Lecithin)	232-249 mg.	241·7 mg.	175-275 mg.
Cholesterol/Phospholipid ratio	53 to 98	72 mg.	74

It is interesting to note that the values for free Cholesterol are lower than the normals obtained in other countries.

The group of " young " atherosclerotis investigated so far, do not show any higher values for the above investigations than the normals. More light will be thrown on this group as a larger series is studied.

SUMMARY.

1. Three groups of patients are being investigated at the present time.

   (a) The hospital class of patient from three of the largest hospitals of Delhi.

   (b) The industrial workers from the Delhi Cloth Mills.

   (c) The rural population of Najafgarh.

Note.—Clerical workers and patients in private practice will be included later in the study when more staff is available to do the work.

A special study is being made of a group of " young " atherosclerotics below the age of 40 years.

2. Cholesterol and Phospholopid estimation are made on all members of the special group and in several of the normals from the industrial and rural populations studied. All cases are interrogated and investigated with the use of two special proforma. A thorough history taking (particularly dietary) and physical examination is done on all of them.

3. It would be premature to draw any conclusions at this stage. The figures worked out so far indicate that:—

   (i) atherosclerosis is responsible for eight per cent of all types of heart disease among the hospital class.

(ii) the incidence is highest at the age group 50-60 among men and women.

(iii) the average intake of animal fat in the diet was 36 gms. in the cases interrogated so far. It is interesting to compare this figure with those for Europe and the U.S.A.

(iv) the blood cholesterol and phospholipid determinations do not show any great deviation for the normal in other countries.

43. Inquiry into the isolation and investigation of *Bacterium coli* strains from (a) new-born babies, (b) children under 12 admitted to the hospital nursery, and (c) pathological material other than fæces under Dr. S. Bhatia at the Lady Hardinge Medical College, New Delhi.

The work on infantile diarrhœas was continued on the same lines as last year. The incidence of diarrhœas due to pathogenic coliform decreased from 24 per cent to 16 per cent of the total number of cases. At no time was there even a minor epidemic.

It was decided to begin work on the pathogenic coli in rectal swabs of new-born infants. The available literature on the intestinal flora of new-born babies (Cruickshank, 1934) gives some information on the subject. It was felt that before undertaking any work on the rôle of potential pathogens in intestinal disorders of the new-born it was essential to have some sort of base line. With this in mind investigation was begun on the normal intestinal flora of the new-born as represented by throat swabs and rectal swabs.

Material.—Only those infants with no abnormality, weighing over 5 lb. and born in normal labour, were investigated during their very short stay in the hospital (i.e., three days). If any of these children developed diarrhœa, temperature or any other disorder, they were not included in the list of normal infants.

Technique.—Rectal and throat swabs were taken daily from these infants—the swabs being moistened with sterile saline before application.

The swabs were plated within 30 minutes of being taken on blood agar, MacConkey and nutrient agar plates. Whole plates were used for rectal swabs and ½ plate for throat swabs. Unfortunately, no anærobic cultures were included.

The plates were examined after 20 hours incubation and the various colonies were investigated by standard techniques.

The results from 50 such infants investigated are tabulated on the opposite page.

None of the coli isolated were amongst the pathogenic strains 055, 0111 and 026. The staphylococci were tested for coagulase using human plasma (Topley and Wilson). It is worth noting that unlike our findings in the cases of diarrhœa due to pathogenic coli (i.e., both rectal swabs and throat swabs showing the presence of coliforms) only a very small number showed coliforms in the throat. Investigations are now being carried out on:—

1. Premature babies.
2. Those new-born infants which show evidence of gastro-intestinal upsets.

Organisms	NUMBER OF CASES, 1ST DAY		NUMBER OF CASES, 2ND DAY		NUMBER OF CASES, 3RD DAY	
	Rectal swabs	Throat swabs	Rectal swabs	Throat swabs	Rectal swabs	Throat swabs
Coliform	18	2	43	1	39	6
Staph. pyogenes	6	3	1	7	5	6
β hæmolyticus Strep.	1	0	0	0	0	0
Strep. pneumoniæ	0	1	0	2	0	5
Strep. fæcalis	1	0	0	0	0	0
Ps. pyocyaneus	7	0	2	0	0	0
B. proteus	0	0	3	0	5	0
Staph. saprophyticus	24	4	6	30	2	35
Ærobic spore bearers	21	5	12	8	8	6

44. Experimental teratogenesis with benzyl alcohol under Dr. P. K. Duraiswami at the Orthopædic Research Laboratory, Safdarjung Hospital, New Delhi.

The main aim of this work is to investigate the mechanism of causation of some human congenital abnormalities on the basis of certain observations made on the analogous developmental anomalies induced experimentally in chick embryos by injecting teratogenic agents such as, benzyl alcohol into the yolk-sacs of hens' eggs at varying intervals after the beginning of incubation.

Injections of 0·01 c.c. to 0·02 c.c. of benzyl alcohol into the yolk-sacs of fertile eggs either before incubation or from the first through the seventh day after the beginning of their incubation give rise to meningoceles, limb deformities, beak defects, localized blebs and generalized œdema.

MATERNITY AND CHILD WELFARE

1. Hæmatological investigations of the new-born babies of anæmic mothers under Dr. R. K. Dutta Chaudhuri at the Chittaranjan Seva Sadan & Cancer Hospital, Calcutta.

It was stated in the last report that the samples of blood from mothers were examined once just before the labour; but since the beginning of this year they are also being investigated at their prenatal period at months interval upto their full term. This study is started in the cases from the antenatal department as soon as they get them registered. Gestation period of the mother and birth-weight of the baby are also being observed so as to find out any correlation with anæmia in the mothers. It may be mentioned here that these additional studies have been taken up as desired by the advisory committee. It may also be mentioned here that in the absence of any agreed maturity weight of the Indian neonate the international standard of maturity 5 lb. 8 oz. (2,500 g.) is taken to be the limiting criteria between the mature and premature babies (Recommendation of Royal College of Obstetricians and Gynæcologists: London, 1948) at present. The degree of severity of anæmia has been classified as mild (8 g. to 9·9 g. per cent) moderate (6 g. to 7·9 g. per cent) and severe (below 6 g. per cent).

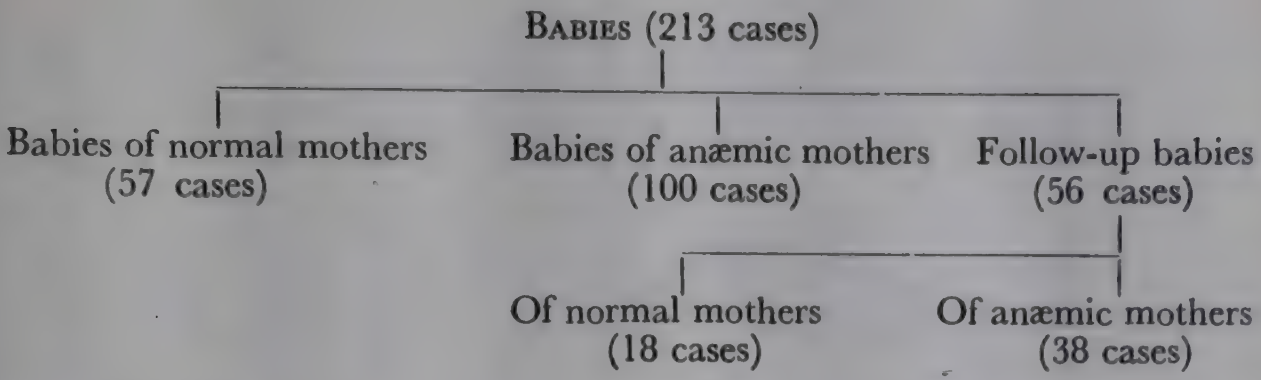
Number of cases studied:—

Total cases: **419**	Mothers	...	206 cases
	Babies	...	213 cases

MOTHERS

- Normal (57)
- Anæmic (114)
  - Hypochromic (52)
  - Macrocytic (28)
    - With Megaloblast (11) ...
      - (*i*) Cases studied 7
      - (*ii*) Mother died during labour 1
      - (*iii*) Others delivered dead baby 3
    - Without Megaloblast (17)
      - (*i*) Cases studied ... 12
      - (*ii*) Mother died ... 1
      - (*iii*) Mother delivered dead baby ... 4
  - †Dimorphic (20)
  - Treated (14)
- Prenatal study (35)

†Dimorphic—(*i*) Cases studied—15. (*ii*) Mother died during labour—2. (*iii*) Mother delivered dead baby—3.


BABIES (213 cases)
Babies of normal mothers (57 cases)
Babies of anæmic mothers (100 cases)
Follow-up babies (56 cases)
Of normal mothers (18 cases)
Of anæmic mothers (38 cases)

Collateral study:

1. Bonemarrow study:—

(*a*) Mothers (44 cases):—

		Case
(*i*) Normal	... ...	1
(*ii*) Microcytic hypo.	...	14
(*iii*) Dimorphic	... ...	12
(*iv*) Macrocytic	... ...	17
(*a*) With megaloblast	...	7
(*b*) Without megaloblast	...	10

(*b*) Babies (12 cases)—Normal mother—1.

Of anæmic mothers:

(*i*) Dimorphic mother	...	6
(*ii*) Megaloblastic	...	3
(*iii*) Microcytic hypochromic	...	2

2. Gastric content analysis of mothers:—20 cases.

(*i*) Normal—5 cases. (*ii*) Hypochromic—8 cases. (*iii*) Dimorphic—4 cases. (*iv*) Macrocytic—3 cases.

OBSERVATIONS.

All the data along with their analysis have been shown in the ten Tables.

The data regarding bilirubin, thrombocyte and osmotic fragility are shown in Tables XI(*a*) and XI(*b*). Analysis is given below:—

Average bilirubin content of blood of mature babies of normal mothers are 2·7 mg. per cent and 4·3 mg. per cent on the first and fourth day, respectively. The babies of anæmic group have average 3·5 mg. per cent and 5·8 mg. per cent of bilirubin in blood of the first day and fourth day, respectively. Premature babies show still higher values, being 3·7 mg. per cent on the first day and 6·8 mg. per cent on the fourth day. This finding is in agreement with previous report.

TABLE I.

Showing the average hæmatological findings of babies of normal birth-weight born of normal mothers and anæmic mothers (according to types) on first day and fourth day.

	NORMAL (43 CASES)		HYPOCHROMIC (38 CASES)		M.A. (8 CASES)		TREATED (6 CASES)	
	1st day	4th day	1st day	4th day	1st day	4th day	1st day	4th day
Hb. g. (per cent)	17·13 (15·6-18·36)	16·05 (14·96-17·51)	17·63 (16·8-18·24)	16·4 (15·26-17·34)	17·08 (14·96-18·04)	16·75 (13·96-18·7)	16·8 (15·81-17·34)	15·75 (14·96-16)
R.b.c. (in million)	5·05 (4·57-5·45)	4·74 (4·53-5·01)	5·12 (4·98-5·5)	4·8 (4·24-5·46)	5·02 (4·33-5·6)	5·04 (4·8-5·19)	5·03 (4·91-5·71)	4·81 (4·41-4·95)
C.v. (per cent)	54·2 (48·0-60·0)	48·0 (42·0-55·0)	54·32 (47·8-61·0)	49·75 (45·0-66·0)	57·0 (54·0-63·0)	50·0 (47·5-54·0)	53·5 (52·2-55·0)	50·81 (47·5-53·2)
M.c.v. (Cμ) ...	107·0 (105-117)	105·2 (100-111)	108·25 (103-111)	102·5 (101-114)	108·4 (105-116)	104·5 (102-111)	107·5 (105-110)	106·0 (104-112)
M.c.h.c. (per cent)	31·4 (30·0-34·3)	32·0 (29·6-34·0)	32·2 (29·8-34·1)	32·35 (30·0-34·8)	31·0 (28·0-35·0)	31·8 (29·0-34·5)	31·25 (30·0-33·0)	32·0 (30·0-33·5)
Reticulocyte (per cent)	5·8 (3·2-6·8)	1·7 (1·0-2·8)	5·2 (2·8-7·0)	1·75 (1·1-2·3)	5·05 (3·4-6·0)	1·6 (0·9-3·0)	5·75 (3·5-6·75)	1·45 (1·2-3·4)
W.b.c. (per thousand)	13·53 (9·9-26·6)	7·64 (5·7-12·3)	12·25 (9·3-19·0)	7·05 (4·5-10·2)	14·23 (10·0-18·0)	8·43 (7·45-9·75)	12·6 (11·1-15·85)	8·71 (6·5-11·2)

Analysis.—

There is no significant difference between the blood condition of babies of normal mothers and that of anæmic mothers on the 1st day and 4th day. Average hæmoglobin value of normal babies on the 1st day is 17·13 g. per cent and on the 4th day it is 16·05 g. per cent. The trend of fall in all the figures of all the groups between 1st day and 4th day is maintained throughout.

TABLE II.

Showing the average hæmatological findings of premature babies of normal mothers and anæmic mothers (according to types) on the first day and fourth day.

	NORMAL (14 CASES)		HYPOCHROMIC (14 CASES)		M.A. (11 CASES)		DIMORPHIC (14 CASES)		TREATED (8 CASES)	
	1st day	4th day	1st day	4th day	1st day	4th day	1st day	4th day	1st day	4th day
Hb. g. (per cent) ...	19·28 (17·8-20·4)	17·02 (16·2-19·2)	19·25 (17·0-20)	17·3 (15·5-18·7)	19·45 (17·3-21·5)	17·34 (15·5-19·3)	19·81 (17·8-22·1)	17·76 (16·3-20)	19·25 (17·6-22·1)	17·16 (16·85-20·0)
R.b.c. (in million) ...	5·36 (5·15-5·79)	5·0 (4·98-5·41)	5·41 (5·2-6·1)	5·0 (4·6-5·6)	5·57 (5·1-6·2)	5·07 (4·8-5·6)	5·55 (5·2-6·4)	4·98 (4·6-5·8)	5·58 (5·2-5·6)	4·83 (4·7-5·2)
C.v. (per cent) ...	61·5 (59-66)	57·6 (54-60)	60·0 (57-65·5)	53·4 (50-55)	60·8 (56-66)	54·5 (50-59)	60·0 (56-67)	54·5 (53-59)	60·5 (56-65)	55·0 (51-62)
M.c.v. (Cμ)	114·5 (109-119)	112·0 (108-118)	109·0 (106-114)	105·5 (112-101)	110·2 (105-115)	104·8 (103-107)	109·3 (105-118)	106·4 (104-111)	110·3 (105-118)	108·5 (100-115)
M.c.h.c. (per cent) ...	30·85 (29·6-33·4)	32·0 (30·0-33·6)	31·36 (29·4-34)	31·7 (29·8-34)	31·5 (30-33)	31·6 (30-34)	32·04 (30·5-35)	32·5 (31-33·2)	31·6 (31-33·2)	32·15 (31-33)
Reticulocyte (per cent)	6·0 (2·1-7·9)	1·9 (1·2-2·0)	5·8 (4·6-6·8)	2·1 (1·2-3·2)	6·6 (3·8-6·9)	2·1 (1·1-2·5)	5·84 (3·2-6·3)	3·24 (0·8-3·9)	5·14 (3·7-4·6)	2·2 (1·9-2·8)
W.b.c. (thousand) ...	13·04 (8·7-16·9)	7·5 (5·5-11·2)	13·85 (10·2-23·0)	7·4 (5·7-10)	12·4 (9·2-15)	8·13 (7·5-10·2)	11·8 (8·8-18·8)	7·97 (4·5-9·5)	12·68 (9·8-18·7)	6·47 (4·6-8·6)

Analysis.—

There is no significant difference between hæmatological condition of premature babies of normal mothers and that of anæmic mothers. The trend of fall remains the same. Average hæmoglobin value of premature babies is 19·38 g. per cent on 1st day and 17·5 g. per cent on the 4th day. Hence, it is about 2 g. per cent and 1 g. per cent, respectively, higher than that of the mature babies on 1st day and 4th day. The rate of fall of hæmoglobin of premature babies on the 4th day is found to be higher than the mature group.

TABLE IIIa.

Showing the average hæmatological findings in babies of normal birth-weight according to severity of anæmia in their mothers on the first day and fourth day.

	FIRST DAY				FOURTH DAY.			
	Normal 43 cases	Mild 26 cases	Moderate 18 cases	Severe 9 cases	Normal	Mild	Moderate	Severe
Hb. g. (per cent) ...	17·13 (15·6-18·36)	17·33 (15·3-18·36)	17·34 (15·13-18·7)	16·05 (15·64-17·68)	16·05 (14·96-17·5)	16·12 (15·3-17·3)	16·52 (15·6-17·6)	14·36 (13·86-15·2)
R.b.c. (in million) ...	5·15 (4·57-5·45)	5·05 (4·8-5·7)	5·15 (4·9-5·37)	4·66 (4·55-5·08)	4·74 (4·53-5·01)	4·81 (4·45-5·13)	4·82 (4·28-5·14)	4·04 (3·98-4·53)
C.v. (per cent) ...	54·2 (48·0-60·0)	54·4 (52-57)	55·06 (54·0-59·0)	50·3 (48·0-54·0)	48·0 (42·0-55·0)	51·0 (47·5-55·0)	50·88 (45·0-54·0)	44·6 (42·7-46·5)

Analysis.—

Table III *a* shows that hæmoglobin level of mature babies of severe group is 16·05 g. per cent on an average on the first day and 14·36 g. per cent on fourth day which is lower than average normal (Tables I and II).

TABLE III*b*.

Showing the average hæmatological findings in premature babies according to severity of anæmia in their mothers on the first day and fourth day.

	FIRST DAY				FOURTH DAY			
	Normal 14 cases	Mild 9 cases	Moderate 13 cases	Severe 26 cases	Normal	Mild	Moderate	Severe
H.b. g. (per cent) ...	19·08 (17·8-20·4)	18·5 (17·37-20·0)	19·3 (17·54-21·42)	18·04 (16·94-21·64)	17·72 (17·2-19·1)	16·8 (16·3-18·53)	18·3 (17·34-20·4)	17·1 (14·26-19·0)
R.b.c. (in million) ...	5·4 (5·15-5·79)	5·34 (5·01-5·9)	5·56 (5·01-6·25)	5·38 (5·0-6·0)	5·23 (4·98-5·41)	4·9 (4·6-5·2)	5·3 (4·6-5·53)	5·05 (4·55-5·3)
C.v. (per cent) ...	61·6 (59·0-66·0)	58·71 (52·0-61·0)	62·03 (55·0-68·0)	59·14 (54·0-65·0)	57·52 (53·0-60·0)	52·2 (46·0-55·0)	54·8 (48·4-58·5)	54·0 (48·0-55·0)

Analysis.—

Hæmoglobin level of premature babies Table III*b* shows no correlation with the severity of anæmia in their mothers. But no conclusion can be drawn at this stage.

Thrombocyte.—There is no significant difference between the thrombocyte count of the babies of normal mothers and that of anæmic mothers. The average count varies from 330,000 to 419,000 per cm. on the first day and 278,000 to 300,000 on the fourth day which is slightly lower than that of the first day.

Osmotic fragility of erythrocytes.—There is no significant difference between the osmotic fragility of erythrocytes of the babies of normal mothers and that of anæmic mothers both on the first day and fourth day. Average osmotic fragility begins at 0·44 to 0·46 per cent of NaCl and completes at 0·30 to 0·32 per cent of NaCl on the first day and fourth day.

Conclusion.—No valid conclusion can be drawn at this stage. It seems, (*i*) that babies of anæmic mothers are not anæmic on the first day and fourth day but they develop anæmia after six months onwards (*vide* Tables I, II and V).

TABLE IV.

Showing the relation of birth-weight of new-born babies with the hæmatological status in their mothers.

	Birth-weight	Normal mothers (57) Normal (57)	ANÆMIC MOTHERS (100 CASES).						Treated (14)
			According to severity			According to type			
			Mild (35)	Moderate (30)	Severe (35)	N.H. (52)	M.A. (19)	D.A. (15)	
Mature	Above 8 lb. ...	3	3	0	0	3	0	0	0
	7 lb. to 7 lb. 15 oz.	6	7	1	1	8	0	0	0
	6 lb. to 6 lb. 15 oz.	23	10	7	6	15	5	0	3
	5 lb. 8 oz. to 5 lb. 15 oz. ...	13	6	9	2	12	3	1	3
Premature	5 lb. to 5 lb. 7 oz.	7	5	4	7	5	5	2	4
	4 lb. to 4 lb. 15 oz.	5	4	6	17	7	6	11	4
	3 lb. to 3 lb. 15 oz.	0	0	3	2	2	0	1	0
	Percentage of mature babies...	78·7	75	56·7	25	73·0	42·1	6·6	42
	Percentage of premature babies	21·3	25	43·3	75	27·0	57·9	93·4	58

Analysis.—

It is found that 93·4 per cent babies of dimorphic mothers and about 75 per cent babies of mothers belonging to severe group of anæmia, are premature, in contrast to 78·7 per cent mature babies of normal mothers, 75 per cent and 56·7 per cent mature babies of mild and moderate group, respectively. This finding indicates that incidence of prematurity and low birth-weight may be related with degree and type of anæmia in mothers. The gestation period of mothers varies from 39 to 40 weeks.

TABLE V.

Average hæmatological findings of follow-up babies of normal and anæmic mothers up to one year.

56 cases.

Age, month		Cases	Hb (g. per cent)	R.b.c. (million)	C.V. (per cent)	M.c.v. (Cμ)	M.c.h.	M.c.h.c. (per cent)	W.b.c. (per 1,000)
1st	N	7	9·77	3·66	34·0	86·5	24·6	30·9	10·5
	A	9	9·4	3·22	31·0	94·0	28·0	30·0	10·9
2nd	N	6	9·52	3·42	30·98	89·25	27·75	30·05	10·3
	A	9	9·24	3·3	30·2	90·52	28·5	30·2	9·7
3rd	N	6	9·25	3·56	30·0	89·0	28·85	30·15	11·7
	A	8	8·66	3·01	28·0	86·6	27·75	30·8	11·8
4th	N	2	9·89	3·81	31·2	82·3	27·1	31·9	11·8
	A	4	9·01	3·58	31·66	83·66	25·56	28·56	14·4
5th	N	2	11·05	3·98	35·0	90·0	27·0	31·6	11·2
	A	5	9·8	3·72	30·0	86·3	26·1	27·7	12·2
6th	N	2	10·03	3·98	31·5	88·0	28·0	31·05	9·3
	A	7	8·35	3·51	29·0	74·0	24·0	26·0	14·3
7th	N	2	10·37	3·83	32·0	83·5	27·3	32·2	...
	A	2	8·8	3·14	29·5	94·0	28·2	29·9	...
8th	N	2	10·2	4·02	31·63	78·5	25·3	31·0	...
	A	2	7·82	3·07	25·65	69·8	21·0	30·0	...
9th	N	1	10·37	4·11	...	...	...	...	...
	A	3	6·8	3·14	27·8	72·6	21·5	29·3	...
10th	N	2	10·2	3·96	32·35	80·6	25·4	31·4	...
	A	2	7·2	3·12	25·29	72·0	21·7	27·7	...
12th	A	1	10·29	4·01	...	...	...	...	...

The findings indicate that babies of anæmic mothers may develop anæmia from 6th month onwards. The hæmoglobin value of the babies of normal mothers is 10·03 g. per cent and that of anæmic mothers being 8·5 g. per cent on an average, at the age of six months. Eight babies of anæmic mothers had to be excluded from the study as they suffered from recurrent attacks of respiratory tract infection.

TABLE VI.

Average bone-marrow findings of mothers and their babies.

	NORMAL (1 CASE)		DIMORPHIC (6 CASES)		MEGALOBLASTIC (3 CASES)		HYPOCHROMIC (2 CASES)	
	Mother	Baby	Mother	Baby	Mother	Baby	Mother	Baby
Hb. (g. per cent) ...	10·2	17·36	2·5 (1·7-3·7)	19·1 (18·5-20)	3·8 (3·4-4·8)	18·8 (17·6-21)	5·34 (4·3-7·02)	17·5 (16·5-19·5)
Total nucleated cells (in thousand) ...	232·0	28·3	156·0 (83·5-193)	31·0 (24-38)	66·0 (49-83)	42·5 (39-45)	234·0	35·2
Normoblast (per cent)	27·5	33·34	41·2 (20·0-59·0)	18·2 (13·0-26·0)	24·8 (14·0-49·0)	15·4 (13·0-17·0)	60·0 (58·0-62·2)	21·0 (15·0-26·0)
Macronormoblast (per cent) ...	0·0	0·0	6·5 (3-11·5)	2·5 (3 cases) (1·8-6·4)	3·18 (2·6-4·6)	0·0	0·0	0·0
Megaloblast (per cent)	0·0	0·0	2·4 (4 cases) (1·2-3·4)	0·0	7 (5·4-9·8)	0·0	0·0	0·0
Giant metamyelo ...	0·0	0·0	Present (3 cases)	Present (2 cases)	Present (2 cases)	Present (2 cases)	0·0	0·0
Granulocyte (per cent)	66·34	48·66	40 (33-56)	55·05 (53-61)	52·3 (47-57)	59·5 (56-64)	31·5 (38-36)	52·0 (48-56)
Macropolycyte ...	0·0	0·0	Present (3 cases)	Present (2 cases)	Present (2 cases)	Present (2 cases)	0·0	0·0
Lymphocyte, per cent	5·66	15·66	7·1 (3·0-11·0)	26·4 (20·6-30·0)	8·4 (6-11·6)	24·2 (18·3-30·0)	5·43 (4·0-6·6)	26·0 (18·0-35·5)

Analysis :

The findings of bone-marrow aspiration of 12 mothers and their babies on the 4th day of their life are shown. Bone-marrow study of 3 new-born babies of dimorphic mothers (Hb.1·7, 2·4, and 3·5 g. per cent) shows preponderance of polychromatic macronormoblast in the smear unlike the other babies. This characteristic feature is also found in the smear (bone-marrow) of their mothers with or without megaloblast. This indicates some correlation of bone-marrow findings of babies with those of mothers. Further study may throw light on this problem.

TABLE VII.

Gastric content analysis of mothers (20 cases).

	C.c. of N/10 NaOH required to neutralize 100 c.c. gastric juice	Normal mothers	Dimorphic mothers	Microcytic hypochromic mothers.	Macrocytic anæmic mothers
Hyperchlorhydria ...	65	*Nil*	*Nil*	*Nil*	*Nil*
Iso-chlorhydria ...	10–45	5	*Nil*	6	2
Hypo-chlorhydria ...	Below 10	*Nil*	2	2	1
Achlorhydria ...	*Nil*	*Nil*	2	*Nil*	*Nil*

Analysis.—

Hypochlorhydria is found in two cases of dimorphic and two cases of hypochromic anæmic mothers and 1 case of macrocytic anæmic mother. Achlorhydria is found in two cases of D. A. group. The rest 13 cases were iso-chlorhydric. No opinion can be given at this stage until further observations are done.

TABLE VIII.

The hæmatological finding of mature babies of normal and anæmic mothers according to the sex of babies.

	NORMAL MOTHERS		ANÆMIC MOTHERS	
	Male (24)	Female (23)	Male (24)	Female (27)
Hb. (in g. per cent)	17·00 (18·3-15·64)	17·3 (18·04-14·96)	17·65 (18·05-15·30)	17·8 (18·3-14·98)
R.b.c. (in million)	4·99 (5·35-4·24)	5·01 (5·35-4·24)	5·1 (4·5-5·6)	5·2 (4·34-5·7)
C.v. (per cent) ...	54·14 (52·0-59·8)	54·5 (52·5-61·0)	56·0 (51·5-63·5)	56·6 (62·0-53·0)
Average birth-weight	6 lb. 12 oz. (5 lb. 12 oz. to 9 lb.)	6 lb. 3 oz. (5 lb. 12 oz. to 7 lb. 14 oz.)	6 lb. 6 oz. (5 lb. 8 oz. to 8 lb.)	6 lb. (5 lb. 8 oz. to 7 lb.)

Analysis.—

There is no significant difference of hæmoglobin level in the male and female babies of normal and anæmic mothers. The birth-weight level indicates that male babies are slightly heavier than female babies. No conclusion can be drawn at this stage of work.

(*ii*) That hæmoglobin values of premature babies are higher than those of the mature babies and rate of fall of hæmoglobin is higher in the premature group on the fourth day (*vide* Tables I and II).

(*iii*) That mature babies of severe anæmic mothers, in contrary to those of normal mothers, show lower values of hæmoglobin on the first day and fourth day of their life (*vide* Table III).

(*iv*) That severe anæmia and dimorphic anæmia of mothers may have some correlation with the incidence of prematurity of the baby (*vide* Table IV).

(*v*) That bilirubin content of premature babies are higher than those of the mature ones (*vide* Table XI).

TABLE IX.

The average hæmatological findings of normal mothers in prenatal period (15 *cases*).

Months	Number of cases	Hb. in g. (per cent)	R.b.c. (in million)	C.V. (per cent)	M.c.v. (Cu)	M.c.h. (rr)	M.c.h.c. (per cent)
5th	4	10·26 (10·17-10·54)	3·33 (3·2-3·46)	32·3 (31·1-33·5)	97·0 (95-99)	30·54 (30-31·08)	31·85 (31·6-32·6)
6th	1	10·54	3·232	33·25	89·0	28·0	31·6
7th	3	10·51 (10·01-10·8)	3·5 (3·3-3·7)	32·3 (31·35-33·25)	92·0 (89-95)	30·6 (28-33·2)	32·0 (31·6-32·4)
8th	6	10·01 (9·5-10·88)	3·1 (3·9-3·6)	31·0 (29·0-33·0)	90·8 (89-91)	28·35 (27-29)	29·5 (28-31)
9th	8	10·68 (10·3-11·02)	3·91 (3·61-3·98)	37·0 (35·39-39)	96·0 (90-98)	29·6 (28·2-31·5)	31·85 (30·7-32)

TABLE X.

The average hæmatological findings of anæmic mothers in prenatal period.

Month	Number of cases	Hb. in g. (per cent)	R.b.c. (in million)	C.v. (per cent)	M.c.v. (Cu)	M.c.h. (rr)	M.c.h.c. (per cent)
7th	8	5·84 (2·55-7·82)	2·9 (0·77-3·3)	20·2 (10-2·6)	74·2 (69-130)	23·0 (19·9-33)	20·3 (18·25-25·6)
8th	9	6·83 (2·38-9·18)	2·38 (1·69-2·9)	21·4 (12-28)	93·6 (61·2-140)	23·54 (21-33)	25·1 (18·6-33)
9th	11	7·1 (5·1-9·1)	2·8 (2·0-3·6)	28·0 (22·8-33)	111·2 (87-122)	30·6 (21-32)	28·3 (24-29)

Analysis.—
(Tables IX and X): From those small number of cases no comment is possible at present.

N.B.—The findings will be expressed in weeks in future instead of months when sufficient number of cases will come under this study.

Table XIa.

The average bilirubin content, thrombocyte and osmotic fragility of red cells of mature babies of normal and anæmic mothers.

	Normal (8)		Hypochromic (7)		M.A. (5)		Treated (3)	
	1st day	4th day	1st day	4th day	1st day	4th day	1st day	4th day
Bilirubin, mg. per cent	2·7 (1·3-3·8)	4·9 (2-5·2)	3·6 (2-5)	5·2 (3·0-8·0)	2·8 (1·4-3·6)	4·5 (3·2-9·5)	2·5 (1·9-6·0)	5·8 (4·0-8·2)
Thrombocyte (per 1,000)	391·0 (254-512)	267·15 (132-422)	362·0 (284-513)	231·15 (205-391)	339·0 (330-432)	303·0 (190-385)	441·0 (411-493)	365·0 (201-463)
Fragility begins at per cent of NaCl	0·45 (0·42-0·46)	0·46 (0·44-0·47)	0·45 (0·43-0·46)	0·40 (0·44-0·48)	0·45 (0·44-0·46)	0·46 (0·44-0·48)	0·44 (0·43-0·46)	0·46 (0·44-0·47)
Completes at per cent of NaCl	0·33 (0·28-0·34)	0·32 (0·30-0·36)	0·32 (0·31-0·36)	0·34 (0·28-0·34)	0·33 (0·33-0·35)	0·30 (0·32-0·36)	0·33 (0·32-0·36)	0·34 (0·32-0·36)

TABLE XI*b*.

The average bilirubin content, thrombocytes and osmotic fragility of red cells of premature babies of normal and anæmic mothers.

	NORMAL (4)		HYPOCHROMIC (6)		M.A. (4)		DIMORPHIC (5)		TREATED (3)	
	1st day	4th day	1st day	4th day	1st day	4th day	1st day	4th day	1st day	4th day
Bilirubin (mg. per cent)	3·5 (1·5-5·0)	6·8 (4·0-8·0)	4·6 (3·0-7·0)	7·2 (6·4-10·0)	5·2 (3·6-6·2)	7·8 (6·3-10)	5·45 (3·4-6·8)	8·0 (5·8-9·5)	...	...
Thrombocyte (per 1,000)	358·0 (212-511)	278·0 (194-313)	419·2 (291-534)	300·0 (234-392)	411·0 (332-466)	332·0 (311-350)	360·0 (292-484)	302·0 (284-396)	412·0 (311-434)	312·0 (265-382)
Fragility begins at per cent of NaCl ...	0·44	0·46	0·46	0·46	0·45	0·46	0·46	0·44	0·44	0·44
Complete set at per cent of NaCl ...	0·30	0·30	0·32	0·34	0·34	0·32	0·34	0·30	0·28	0·32

(*vi*) That bilirubin content of blood rises on the fourth day in all cases without any significant change in the osmotic fragility of erythrocytes (*vide* Table XI).

(*vii*) That the studies of bone-marrow of both mothers and their babies done so far (though small in number) may throw some significant light on the problem under investigation (*vide* Table VI).

2. Inquiry on the estimation of œstrogen content of urine in toxæmias of pregnancy with special reference to etiology and prognosis under Dr. S. Mitra, S. Basu *et al* at the Chittaranjan Cancer Hospital, Calcutta.

Uptil now 100 normal pregnancy cases and 50 cases of toxæmia of late pregnancy have been assayed. The method used was the modified technique of Stevenson and Marrian for the chemical assay of œstrogens from urine.

Technique.—The three fractions of œstrogens, i.e., œstradiol, œstrone and œstriol can be separately estimated by following the colorimetric procedure. The process can be mentioned briefly into the following steps:—

Step A	...	Open flask acid hydrolysis.
Step B	...	Extraction of total œstrogen from acid hydrolysed urine.
Step C	...	Separation of œstriol from œstrone and œstradiol in urine extracts.
Step D	...	Separation of œstradiol from œstrone by Girard's Reactif ' T '.
Step E	...	Colorimetric estimation of the three fractions by Kober's reaction.

Results.—The weeks of pregnancy have been divided into following groups:—

(*a*) From early weeks up to 30th week.
(*b*) 31 to 32 weeks.
(*c*) 33 to 34 ,,
(*d*) 35 to 36 ,,
(*e*) 37 to 38 ,,
(*f*) 39 to 40 ,,

As the cases in early weeks of pregnancy were not available in a considerable number, they have been taken into one group and the average result of these has been considered.

In each week at least seven to eight samples have been assayed and the average result is taken into consideration. All Tables and graphs are appended herewith to make a complete study.

GRAPH 1.	Excretion of total œstrogen in normal and toxæmia of pregnancy.
GRAPH 2.	Excretion of œstriol in normal and toxæmia of pregnancy.
GRAPH 3.	Excretion of œstradiol and œstrone in normal and toxæmia of pregnancy.

TABLE I.

Œstrogen excretion in normal pregnancy. (100 cases).

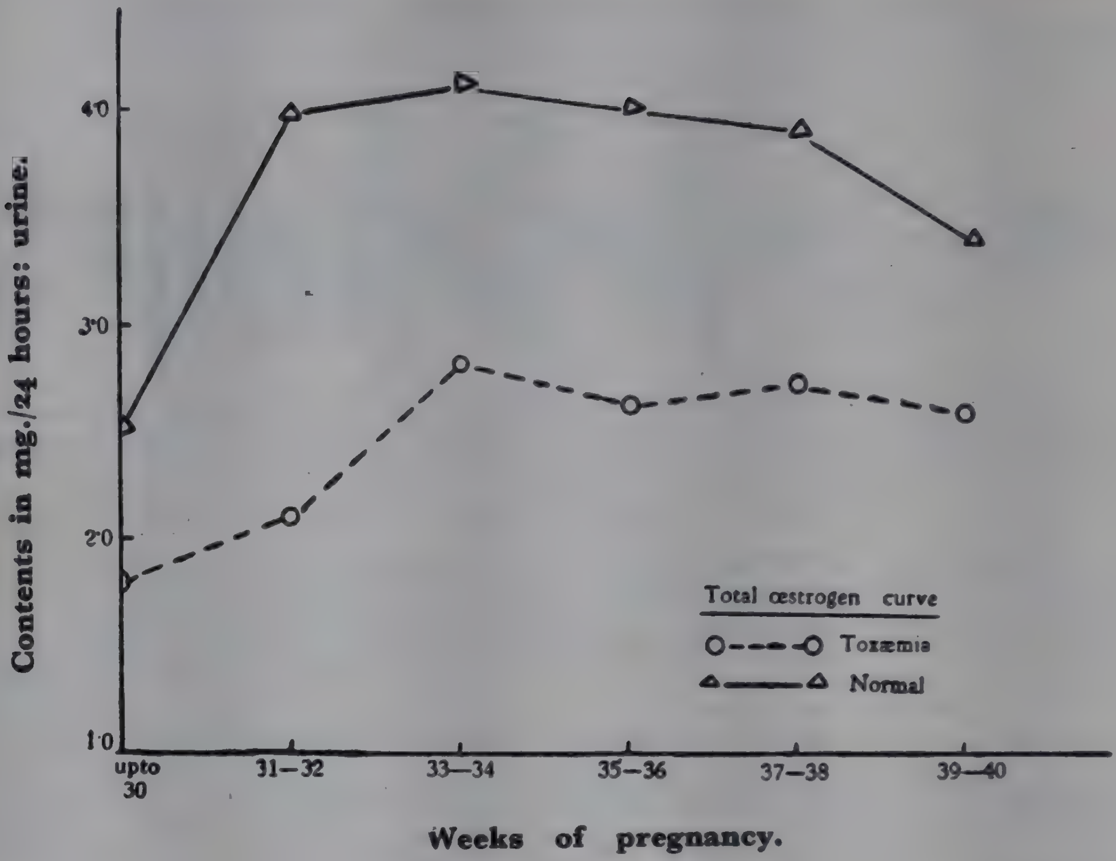
Weeks of pregnancy	Number of cases	Average excretion of œstriol/24 hours in mg.	Average excretion of œstrone/24 hours in mg.	Average excretion of œstradiol/24 hours in mg.	Total average excretion of œstrogen/24 hours in mg.	Rat units (approximately)
Below to 30 weeks	25	1·53	·48	·57	2·58	4,250
31 to 32 ...	10	3·14	·38	·47	3·99	6,630
33 to 34	10	3·59	·23	·30	4·12	6,970
35 to 36	10	3·06	·37	·60	4·03	6,800
37 to 38	20	2·90	·54	·47	3·91	6,630
39 to 40 ...	25	2·75	·24	·42	3·41	5,780

TABLE II.

Œstrogen excretion in toxæmia of pregnancy. (50 cases).

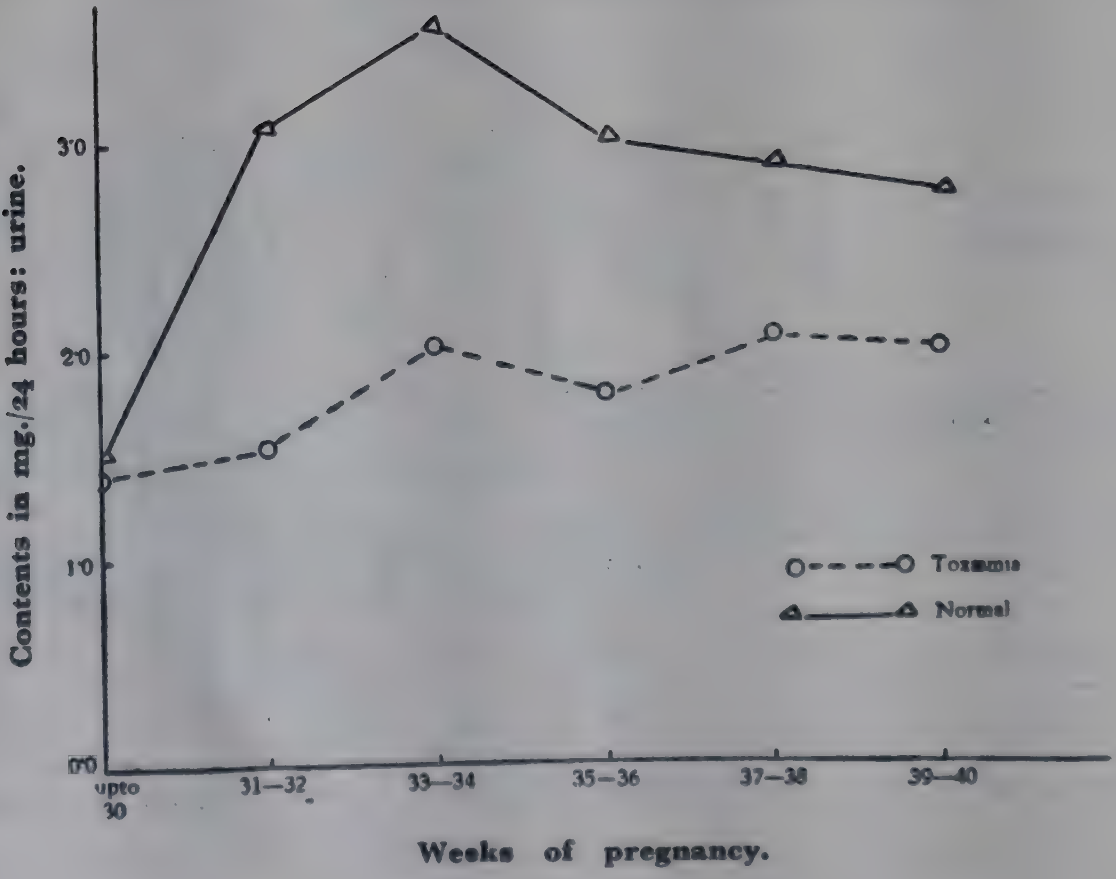
Weeks of pregnancy	Number of cases	Average excretion of œstriol/24 hours in mg.	Average excretion of œstrone/24 hours in mg.	Average excretion of œstradiol/24 hours in mg.	Total average excretion of œstrogen/24 hours in mg.	Rat units (approximately)
Below to 30 weeks	8	1·44	·12	·33	1·80	2,960
31 to 32 ...	8	1·54	·46	·13	2·13	3,570
33 to 34 ...	7	2·05	·34	·47	2·86	4,660
35 to 36 ...	8	1·80	·41	·45	2·66	3,910
37 to 38 ...	10	2·08	·27	·41	2·76	4,590
39 to 40 ...	9	2·00	·20	·40	2·60	4,420

GRAPH 1.



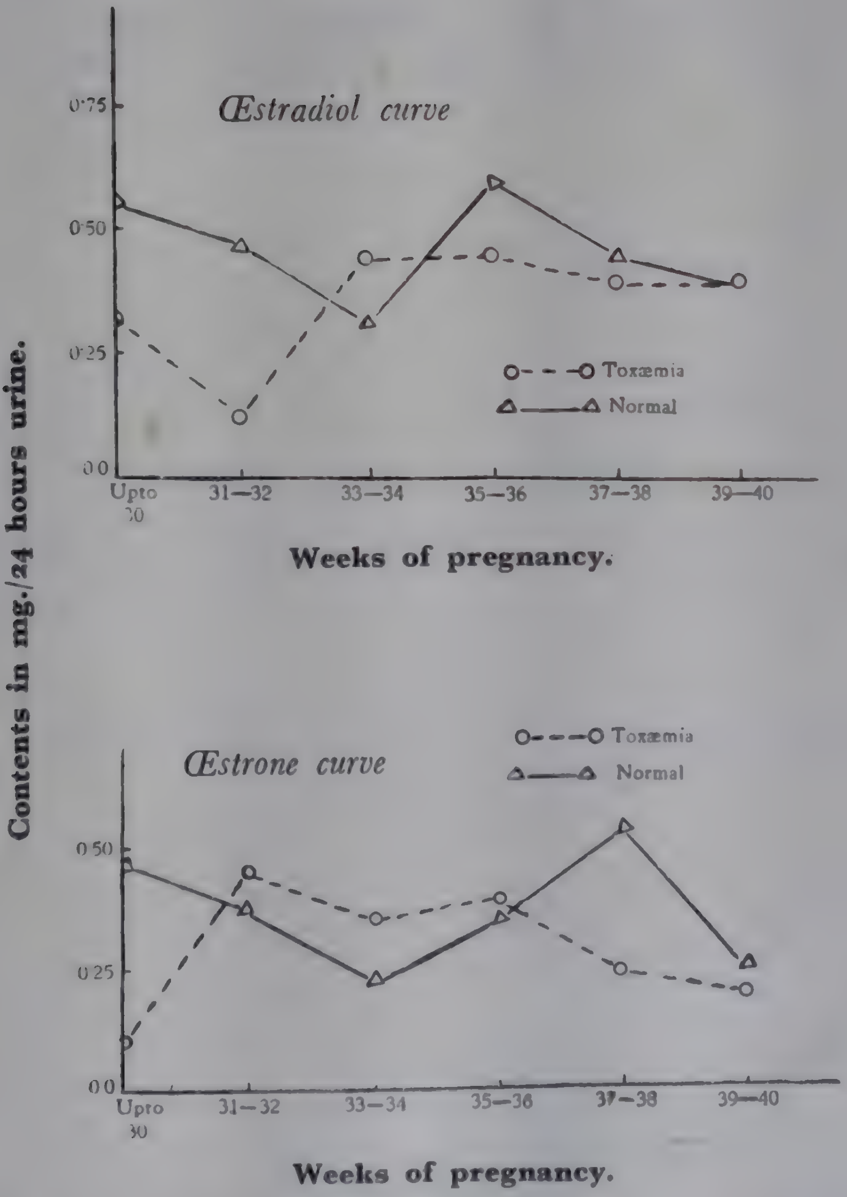
GRAPH 2.

Oestriol curve.



Discussion.—It has observed that there is more or less a steady rise of œstrogen level with the progress of pregnancy followed by a fall prior to labour in normal cases. In toxæmic cases there is definitely a low excretion all through the pregnancy although maintaining a similar pattern of rise and fall. Our results compare favourably with the previous workers both as regards the total œstrogens as well as the different fractions. As is expected it has been observed that when œstriol excretion is high, the excretion of œstradiol and œstrone is low and vice versa. This is probably an index of the metabolism of œstrogen at a particular time in a particular individual. The low excretion of œstrogens in toxæmic cases is probably due to degeneration of syncitial cells or due to failure of utilization of chorionic gonadotropins (Smiths).

GRAPH 3.



It can thus be assumed that in a case of toxæmia, a condition of syncitio-steroid aberration exists resulting in the breakdown of the correlation of and synergism between the steroids and chorionic gonadotropins.

ŒSTROGEN ASSAY BY CHROMATOGRAPHY.

We have been recently working on chromatographic methods for œstrogen assay. We are following Braunsberg's method for fractionation of the three fractions of œstrogens.

Technique.—The technique may be briefly summarized as follows:—

The stationary phase is 3·1 N aqueous sodium hydroxide held by cellite 535 as the supporting medium. Œstrone and œstradiol are eluted with benzene: petroleum ether, b.p. 60 to 80 degree centigrade, 4:1 (v/v) and œstriol with a 17:3 chloroform : butanol mixture.

The diagrams of the elution pattern of the standard solution of the three fractions and in the urine of normal and toxæmia of pregnancy cases are given below to make a complete study:—

Graph 1—Chromatograph of the pure samples of oestrogens. (Standard)

Graph 2—Chromatograph of œstrogen in normal pregnancy.

Graph 3—Chromatograph of œstrogen in toxæmia of pregnancy. Uptil now we have done 20 cases, the results of which are shown in Table IV.

TABLE IV.

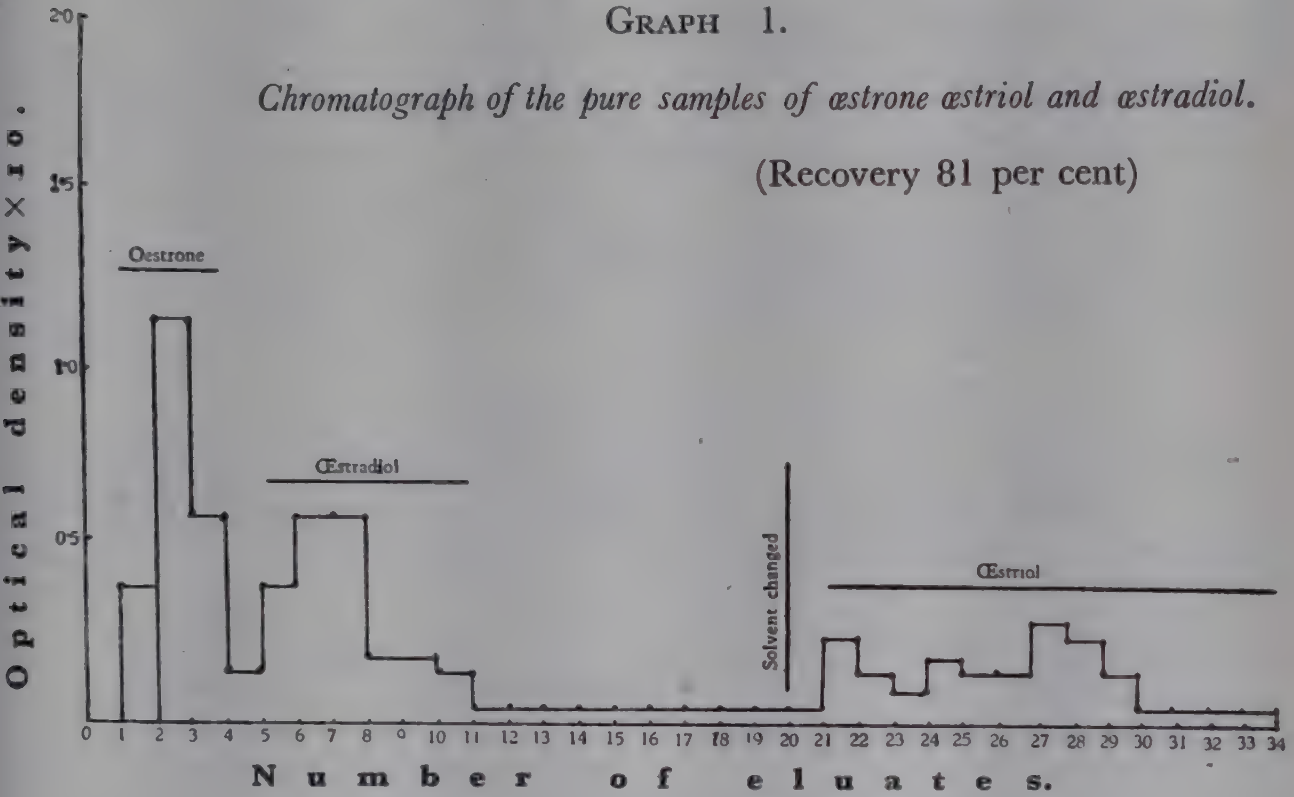
In normal pregnancy.

Weeks of pregnancy	Number of cases	Average excretion of œstrogens, mg./24 hours
From early to 30	3	1·344
31 to 32	2	1·600
33 to 34	1	1·280
35 to 36	2	2·540
37 to 38	...	No case obtained
39 to 40	3	2·552

GRAPH 1.

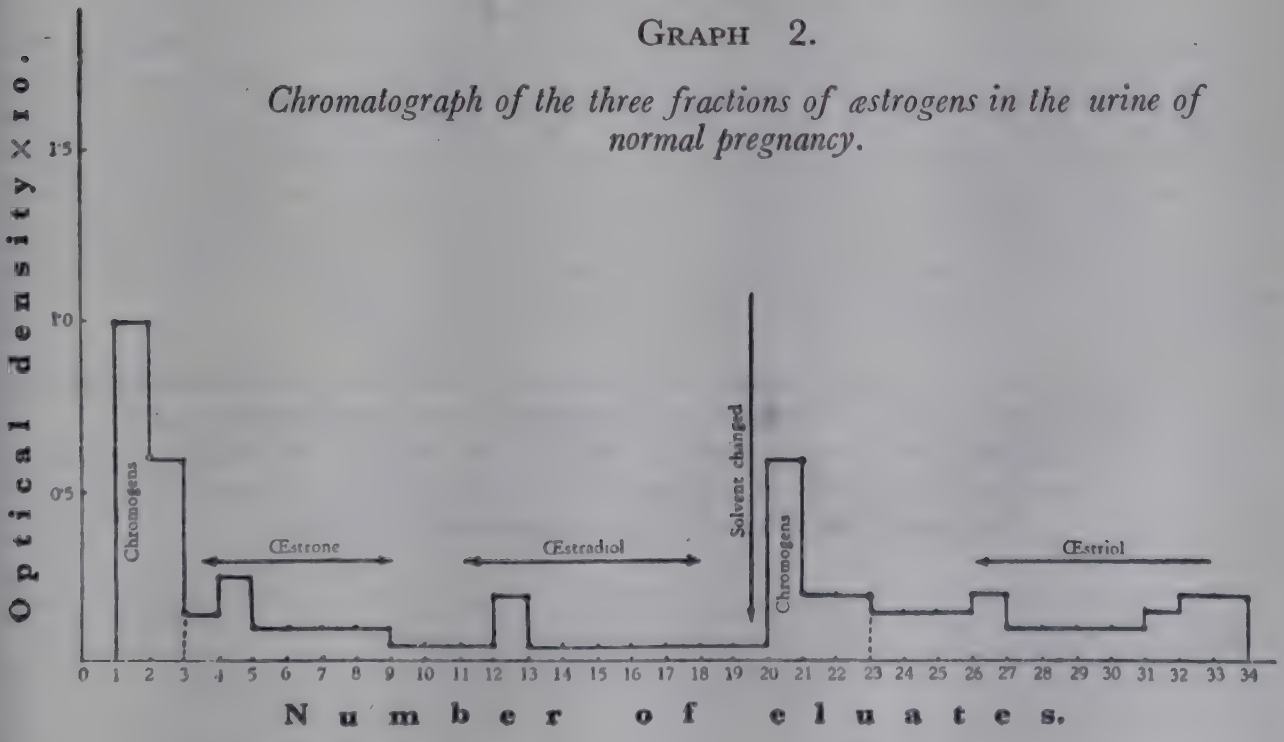
Chromatograph of the pure samples of œstrone œstriol and œstradiol.

(Recovery 81 per cent)

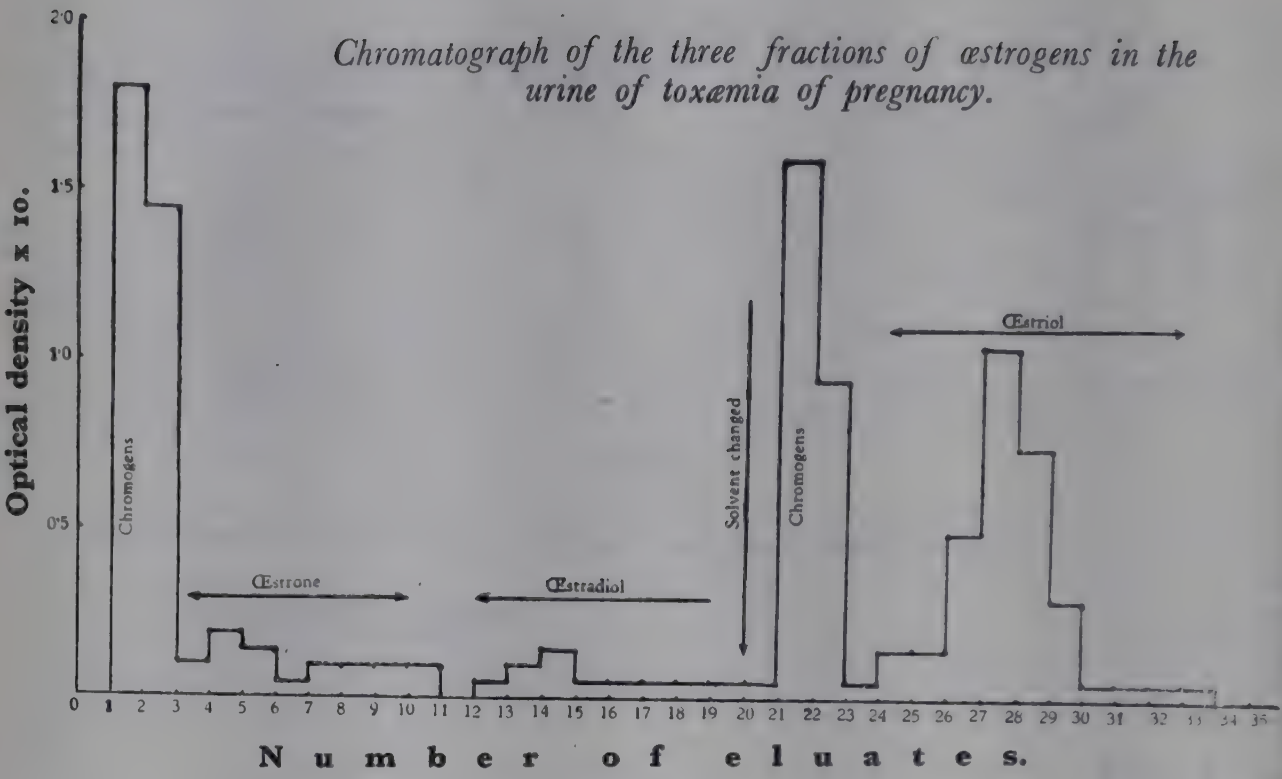


GRAPH 2.

Chromatograph of the three fractions of æstrogens in the urine of normal pregnancy.



GRAPH 3.



Chromatograph of the three fractions of œstrogens in the urine of toxæmia of pregnancy.

TABLE V.

In toxæmia of pregnancy.

Weeks of pregnancy	Number of cases	Average excretion of oestrogen, mg./24 hours
From early to 30	2	1·414
31 to 32	2	1·659
33 to 34	...	No case obtained.
35 to 36	3	1·503
37 to 38	...	No case obtained.
39 to 40	2	·916

Discussion.—From Graphs 1, 2 and 3, it is clearly understood that there is less excretion of œstrogens in toxæmia of pregnancy. In pregnancy urine the presence of chromogenic substances and their interference in the estimating procedure has been shown in Graphs 1 and 2. There may, however, be a slight variation in the picture of chromatograph with the variation of temperature as has been found in the temperature variation during the run of the experiment. We exactly tried to find out the perfection of the technique for which we did the recovery experiment and it has been shown in the graph that the recovery is 81 per cent.

3. Study of nutritional status of expectant mothers with particular reference to the incidence of toxæmias of pregnancy and anæmias in pregnancy in different parts of India under Dr. R. K. K. Tampan at the Government Hospital for Women & Children, Madras.

Women 20 to 28 weeks pregnant are selected for the study, two criteria being used for selection of cases:—

(*i*) The patient should be able to give her correct address.

(*ii*) She should be sure of coming to this hospital for the delivery.

It is necessary to insist on these two points, as follow-up, which is difficult now, would become almost impossible otherwise.

At the patient's first visit, the proforma provided is filled up. A history of the past health and obstetric and diet histories are recorded. A complete physical examination is made including height, weight and intercristal diameter. Any manifestation of nutritional deficiency and its degree, as can be clinically assessed, is noted. The blood pressure is recorded and the urine is tested for albumin. Hæmoglobin is estimated by the acid-hæmatin method with a Hellig's scale. A sample of blood (10 c.c.) is taken for the VDRL test, and for estimation of serum proteins and serum calcium.

Considerable time is spent in impressing on the patient the need for periodic visits at monthly intervals. All patients are advised to add milk and fruits to their diet (advice that is rarely followed). Multivitamin and polyhæmin tablets (Ferri. sulph. 10 grs./day) are prescribed as a routine, but never taken for more than two days, due to the inconvenience of having to travel long distances to the hospital.

At the monthly visits, the clinical examination is repeated, B.P. and weight are recorded, urine tested for albumin, hæmoglobin estimated and diet history enquired into.

Biochemical estimations are repeated once in three months.

Results.—Ninety patients have been examined from 10-8-55 to 29-10-55. Sixty-nine of these patients were due for revisits. Twelve patients have reported voluntarily for follow-up. Letters were sent to all the rest and 19 more responded. Thirty-eight patients have not reported at all. Five patients have come for a second re-visit.

As none of the patients has completed full term, no detailed analysis is attempted at this stage and only general impressions are given.

Social status.—The majority of the patients belonged to the poor and lower middle class families.

TABLE I.

Family income in rupees per mensem	Number of patients	Percentage
Less than 50	45	50
50 to 100	23	26
100 to 200	14	16
200 to 500	7	8
Totals ...	89	100

Diet.—The quality of the diet was found to be very poor in the majority of cases. Rice, and a small quantity of vegetables every day, dhal on alternate days, with meat or fish once a week was the average pattern of diet. Milk and eggs were rarely included except by an occasional patient of higher economic status. Advice given to improve the diet by addition of milk, eggs and fruits was never followed (as few of the patients could afford these) and an enquiry at re-visit showed little or no alteration in the diet.

Clinical findings.—Most of the patients were underweight and often showed evidence of nutritional deficiencies. Vitamin A deficiency, as manifested by discolouration of the conjunctiva, varying from patchy pigmentation to a generalized muddy appearance, was found in 67 per cent of cases. Twenty per cent had xerosis of the conjunctiva (Bitot's spots). The cornea was not affected in any of the cases.

Riboflavin deficiency manifested as, angular stomatitis, sometimes accompanied by glossitis was found in 20 per cent of the patients. Six patients who did not have angular stomatitis at the first visit had developed it by the second visit.

Fæces examination.—In spite of repeated instructions, only 37 patients brought a sample of stool for examination.

Two of these patients had amœbic infection, nine had ascariasis, two had oxyuriasis and three had ankylostoma infection.

TABLE II.

Hæmoglobin estimation.

Hæmoglobin in g. per cent	Number of patients	Per cent of patients
More than 12	7	8
10 to 12 ...	56	62
8 to 10 ...	22	24
Less than 8 ...	5	6
Totals ...	90	100

The values were obtained at the first visit of all the patients. At the re-visits, a steady fall in the hæmoglobin level was noticed, and borderline degrees of anæmia were quite common.

Blood chemistry.—Serum-calcium levels varied from 8·5 mg. to 12·8 mg. per cent, average value being 10·2 mg. per cent. Total serum proteins ranged from 5·06 mg. to 7·35 mg. per cent with a mean of 6·15 g. per cent. The albumin-globulin ratio was reduced in most cases, but there was no reversal in any of the cases.

The trends of serum calcium and serum proteins, as pregnancy progressed, have not been studied as three months have not elapsed since the investigations began.

Difficulties encountered.

(1) *Selection of cases.*—The majority of patients attending the antenatal clinic are either in the first or in the late part of third trimester of pregnancy. The latter cannot be included in this series. The former were not selected for two reasons:—

(*a*) They might abort and no follow-up would then be possible.

(*b*) In spite of explaining to each patient the importance of monthly re-visits, follow-up, as mentioned above, has not been satisfactory, and a long interval before delivery would make it freely random.

(2) *Diet history.*—Various methods have been adopted in other nutrition studies to assess the diet of a patient. The most accurate is the individual weighing method. Every article of food taken by the patient is weighed for a number of days, and the average consumption per day calculated. Economic reasons and illiteracy do not make this method practicable here.

The next preferable method would be to weigh the total amount of each ingredient of food consumed by all the members of the family, and to calculate the individual intake according to the international scale of family coefficients. This method would require a large number of workers with facilities for house-visits and weighing.

The questionnaire method has its merits in that it is less cumbersome. A reasonable degree of accuracy can be achieved by questioning the subject every day, for at least a week, recording each item, and subsequently analysing the ingredients of the diet. Diet analysis of this magnitude would not be feasible under the present circumstances.

The method followed for the present survey will give approximate values only. The patients are able to give only a rough idea of the total quantity of food purchased for the family for varying periods. The quantity is described in weight measures, by bulk and very often according to the cost. It is not unusual for patients to say that she buys one anna worth of oil, two annas worth of vegetables and four annas worth of fish in a day. This method undoubtedly gives us an idea of the gross inadequacy of the diet in general; but where accurate food values are to be calculated to show the relationship, if any, of diet to pregnancy complications, this history will not be of much help.

(3) *Co-operation of patients and follow-up.*—This is perhaps the worst handicap in work concerned chiefly with out-patients. Only 37 of the 90 patients could be persuaded to bring a specimen of stool for examination. As mentioned earlier only 12 cases reported voluntarily for a re-visit. After considerable time and money was spent in writing letters to the rest, 19 more turned up. Thirty-eight patients have not come back. As no social worker has been appointed for this scheme, no home contact is possible. Adequate follow-up thus becomes very difficult.

NUTRITION STUDIES IN PREGNANCY.

It is believed that the diet of a pregnant woman has a profound influence on the health of the mother and the development and progress of her infant. Various workers in Western countries have studied the problem in its various aspects with widely varying results. Some have used the method of determining the value of dietary supplements. Others have made extensive nutrition surveys and studied the relationship of diet and nutritional status to the course of pregnancy labour, the puerperium and the health and progress of the infant.

In the year 1938-39, the People's League of Health carried out an extensive experiment in ten London hospitals, the results of which were published in 1946. Alternate patients enrolled at the antenatal clinic were given dietary supplements. The others were used as controls. These groups were again subdivided according to age and parity for purposes of comparison. All women who were not in good health or who were more than 24 weeks pregnant were excluded from the experiment. The daily supplements given to the 'treated group' were as follows:—

Saccharated iron carbonate gr. 18; calcium lactate gr. 30; minute quantities of iodine, maganese, copper; adsorbate of vitamin B, containing all factors of the B complex; group 15 (B content standardized to 200 I.U./I); vitamin C 100 mg.; halibut liver oil 6 minims.

At the conclusion of the experiment the record of 5,022 women were analysed and the results were briefly as follows:—

(1) No significant influence was found on the character or duration of labour, on the birth-weights of the infants, or on the incidence of fever and sepsis after child-birth.

(2) The incidence of premature births among primiparæ was 23·9 per cent for the controls, and 20 per cent for the treated cases, the figures for multiparæ being 24·2 per cent and 20·1 per cent. Although there is an undoubted decrease for the treated cases, the figure is still too high.

(3) *Toxæmia of pregnancy.*—The incidence of toxæmia in the treated primigravidæ was 5·4 per cent and in the controls 7·4 per cent. In the parous women the incidence was 3·6 per cent in the treated group and 5·2 per cent in the controls. It was, therefore, claimed that the treated group was protected in a ratio of almost 30 per cent. The duration of the treatment, however, had no effect.

Ebbs, Tisdall and Scott (1941) carried out a similar experiment in Toronto General Hospital, involving 380 pregnant women. These women were divided into three groups: (1) Those of poor economic status who served as controls, (2) those of poor economic status, whose diet was supplemented for the purpose of the experiment, and (3) those of higher economic status who were educated to take a satisfactory diet. The supplements given were as follows: 30 ounces of milk; one egg, one orange, one riosterol capsule containing 2,000 I.U. of vitamin D, and once a week, 16 oz. of factory canned tomatoes and ½ lb. of cheese and dried wheat germ containing malt and added iron.

As a result of this experiment, these workers claim that the incidence of miscarriages, still-births and premature births was significantly higher among the control group. The mothers on a good or supplemented diet had few complications during pregnancy and few difficulties during labour than the control group. The incidence of illness in the babies up to the age of six months and the number of deaths was greater in the control group. The total number, however, involved are too small for chance to be eliminated.

Williams and Fralin (1942) investigated the diets of 514 pregnant women. They found that errors in food habits and inadequate diets were the rule. The caloric, carbohydrate and fat intake were unaffected by income. The intake of vitamin A, B, B_2 and C rose with income. Protein, calcium and iron intake showed a slight rise with income. No positive relationship was found between dietary adequacy and complications of pregnancy and child-birth. There was no evidence that toxæmia occurred in the presence of insufficient protein, vitamin A or B. Protein or vitamin-A deficiency did not seem to be responsible for premature labours. The number of neonatal deaths and still-births was too small to demonstrate any relationship. With regard to puerperal morbidity the diets of these women did not differ from the remainder in respect to their protein, vitamin A or C intake.

Protein intake had no influence on the ability to nurse the baby. The diet of the 250 women who had no complications at any stage showed no variation from the entire group. A point of interest noted was that 84 per cent of those who had nausea and vomiting in early pregnancy had a low intake of vitamin B_1.

Baird (1945) in Scotland has shown that among the women in the lower socio-economic groups, the incidence of prematurity was twice, the still-birth rate three times and the neonatal mortality rate four times, the comparable rates found in the women of the upper economic groups. Baird discussed the fact that the still-birth and neonatal death rates appear to be controlled by social conditions which operate through the mother. The still-birth rate in England, Wales and Scotland fell during the war for all age groups and parities. He considered it probable that the factor which was operating on a national scale, was the improvement in the diets of the poorer women.

Smith (1947) studied the case records of 2,254 women in Holland who had been pregnant or had delivered during the severe war famine of 1944-45. He noted the following effects of the severe undernutrition: (1) Amenorrhœa and infertility were so common as to reduce strikingly the expected number of births. But age and parity ratios were not affected. (2) Infants conceived before the famine months but borne during these months were significantly below the expected weight and length demonstrating the effect of maternal nutrition during the last half or last trimester of pregnancy on fœtal development. (3) Prematurity and congenital malformations were slightly but not significantly increased. (4) No effect was noted upon the incidence of still-birth, neonatal death, or lactation. (5) One surprising fact which emerged was that pre-eclamptic toxæmia and eclampsia decreased, the figures for the former being 3·2 per cent before the war and 1·9 per cent during the famine. Smith (*loc. cit.*) comments that Dutch women had been reasonably well fed until subjected to a brief period of acute general undernutrition. Probably, acute undernutrition may result in one way, while chronic malnutrition may result in another.

Burke and her associates studied a group of 216 women from the antenatal clinic of Boston Lying-in Hospital. Detailed nutrition histories were obtained at intervals during pregnancy. An overall relationship was found to exist between a good or excellent diet during pregnancy and good physical condition of the infant at birth. All still-born infants, except one who died in the neonatal period, all except one of the premature infants, most of the infants with major congenital defects and all the infants considered to be functionally immature were born to women in the poorest diet group. In contrast 94 per cent of the infants born to mothers on good or excellent diets during pregnancy were in good or excellent condition at birth. The relationship between the general dietary habits and the incidence of toxæmia was significant. There was no toxæmia among women whose diets were good or excellent, while 44 per cent of those in the poorest group had toxæmia. No significant relationship was found between the antepartum diet and the length of labour. There were, however, many more difficult types of deliveries in the poorest diet group despite the fact that these infants at birth averaged 3 lb. lighter in weight, and several cm. shorter in length than the infants of mothers whose diets were good or excellent. (A rating scale was used in evaluating the diet. Each nutrient was rated excellent, good, fair, poor or very poor according to the range in which the calculated value of the average intake fell).

In a later series of 284 cases she confirmed her observations. Data regarding 53 matched pairs of sibbling showed improvement of the condition of the infant with improvement in the maternal diet and deterioration of condition of the infants with impoverishment of the maternal diet, in all the cases. In this series of matched sibblings each mother was her own control and hereditary and environmental influences were eliminated.

Hobson, Lewis and Woodman (1949) made a survey of the nutrition of 111 primigravidæ. The survey estimated the total food intake over a period of one week—all food taken being weighed and recorded by the patient and supervised by the health visitor. Blood samples were also taken for biochemical and hæmatological examination. It was found that the intake of calories (2,400) and protein (1·5 kg.) was satisfactory. The average iron intake was fairly high (14 mg.), calcium intake (1·2 g.) and that of vitamin B_1 (1·25 mg.), B_2 (1·7 mg.), niacin (10 mg.) and vitamin C (81 mg.) was below the standard recommended by the U.S. National Research Council. No correlation was found between variation in protein intake and level of serum proteins. There was no fall in serum proteins or albumin but there was a rise in the globulin fraction. There was a progressive fall in hæmoglobin percentage as pregnancy advanced but the m.c.h.c. was not altered. No correlation was found between iron intake and hæmoglobin levels, probably because, the dietary intakes were, on the whole, high. Erythrocyte sedimentation rate was increased in normal pregnancy and was of clinical importance only if it was over 30 mm./hour. It had no relation to diet. There were 17 cases of toxæmia, many of whom were on quite good diets. Only one did not take the vitamin supplement daily, the great majority had a poor intake of vitamin B_1 and nicotinic acid. But two had high intakes of B_1. Nicotinic-acid intake of toxæmia patients was 8·3 mg. compared to 10·3 mg. of the 94 non-toxæmic. He considered it likely that there was some relationship between vitamin-B deficiency and toxæmia of pregnancy.

Sprert, Graff and Graff (1951) studied the relation of nutrition to premature labour. Having eliminated all the known causes of prematurity, they included in their study 70 women who had premature labour due to unknown causes. An intensive nutrition study was made of these women and of 67 women who had normal full term deliveries. This included diet histories, estimation of serum protein, serum amino-acid nitrogen, red cell volume, hæmoglobin, serum alkphosphatase, serum vitamin A, serum carotene, serum ascorbic acid and whole blood ascorbic acid. No significant differences were found. This study, therefore, did not support the theory that nutritional deficiency is a common cause of premature labour.

Scrimshaw (1951) in an evaluation of nutrition in pregnancy, carried out an investigation of 10,000 canal zone and Panama women. He found that the incidence of pre-eclampsia and eclampsia was not significantly different in the high income and low income groups. In the West Indian Negroes who had a poor diet, a much higher incidence was found though the diet of this group was similar to the diet of the low income Panama group. The Negroes were, however, socially suppressed, and inclined to be of a sullen temperament consequently. And as it was found that the diets of women with pre-eclampsia were not lower in protein and poorer in general than those whose pregnancies were uneventful. Scrimshaw suggests that social psychological factors influence the incidence, not only of essential hypertension but also of pre-eclampsia.

The Vanderbilt co-operative study of nutrition carried out an extensive investigation programme. All patients who attended the antenatal clinic of Vanderbilt University Hospital between 1945 and 1950 were entered for the study. In all, 2,046 patients were seen once during each trimester and once six weeks postpartum, a nutritional evaluation of each patient being made by physical examination for evidence of deficiencies, biochemical assessment of body fluids, for protein, vitamins and others and nutritional dietary records for seven-day periods. It was found that in general the average nutritional intake fell below the dietary allowances recommended by the U.S. National Research Council. Hæmatological and biochemical changes noted were: (*i*) As pregnancy advanced there was a decrease in the hæmoglobin concentration, r.b.c. count, and p.c.v., the blood picture remaining normochromic, normocytic. (*ii*) A decrease in the serum total protein and albumin was noted (Hobson) consistent with the increased plasma volume or hæmodilution. All these values returned to the initial values at the postpartum check up. (*iii*) Serum vitamin-A concentration decreased during pregnancy, the level rising again postpartum. (*iv*) Serum carotene behaved in the opposite manner. (*v*) Serum ascorbic acid decreased slightly during gestation, a sharp fall occurring postpartum in women who breastfed their infants (0·2 mg./100 c.c.). (*vi*) A slight fall occurred in thiamine and riboflavin excretions during pregnancy but N. methyl-nicotinomide excretion rose from 6·6 mg. to 9·6 mg./2 hour. The significance is not understood. No physical evidence of frank deficiency disease was found.

Relationship of obstetric performance to nutrition.—Only the relation of the more important complications to nutrition will be considered here.

Anæmia.—The anæmia developing during the gestational or puerperal period was hypochromic and could usually be related to complications associated with excessive blood loss. The group of patients who developed this anæmia had low average intakes of iron (12 mg.) and vitamin C (46 mg.) associated generally with low hæmatological and other serum determinations.

Toxæmia.—The average nutrient intake of these women tended to be lower than the total study group, consistently for niacin. This was associated with a smaller than expected excretion of N. methylnicotinamide. During the second trimester prior to the recognition of toxæmia, this group had an elevation of the total serum proteins and albumin, while other serum or blood levels showed that there was no hæmo-concentration. The disturbed water balance and metabolic changes seen after the development of the toxæmia state result in changes in the serum-protein concentration which have led to the belief that toxæmia is due to protein depletion.

Puerperal complications.—In those patients who developed puerperal complications or fever, there was a decrease in the average level of serum vitamin A and hæmatological values, throughout gestation, but the significance of the difference in vitamin A is questionable as it always remained in excess of 100 I.U./100 c.c.

Still-birth and neonatal deaths.—No significant difference was noted.

Premature infants.—Apart from a decrease of serum ascorbic acid, the remainder of the blood determinations showed slightly elevated values. No relation could, therefore, be drawn between maternal nutrition and prematurity.

In our country, the nutrition of pregnant women has not attracted special attention until now, probably because malnutrition has been so common and widespread.

NUTRITIONAL REQUIREMENTS IN PREGNANCY AND LACTATION.

Whether or not the nutritional status of a woman has a direct bearing on the outcome of her pregnancy, it remains an undisputed fact, that a healthy mother has a better chance of a successful pregnancy and of a healthy baby and that an ample diet will contribute towards that health.

The National Research Council of the U.S.A. has recommended the following intakes for the pregnant and the lactating women:—

	Non-pregnant	Pregnant 4th to 9th month	Lactating
Calories	2,100	2,500	3,000
Proteins, g.	60	85	100
Calcium, g.	·8	1·5	2
Iron, mg.	12	12	15
Vitamin A (I.U).	5,000	6,000	8,000
Ascorbic acid, mg.	70	100	150
Thiamine, mg.	1·1	1·8	2
B_2, mg.	1·5	2·5	3
Nicotinic acid, mg.	11	18	20
Vitamin D (I.U.)	...	400—800	400—800

These allowances represent, according to some workers, the minimum required. Others regard them as well above the safety margin.

Calories.—The basal metabolic rate of a pregnant woman shows little change until the 6th or 7th month, when the fœtus begins to show a rapid gain in weight. It has been estimated that this increase is entirely due to the basal metabolic rate of the fœtus and to some extent, to the increased mass of maternal structures, and the metabolic rate of the mother remaining unaeltred. Moreover, the physical activities of a pregnant woman are curtailed. A 20 per cent increase in caloric intake should then be considered quite sufficient to meet the needs of pregnancy.

Dieckmann (1945) considers that woman who begins pregnancy with a normal weight should not gain more than 7 kg. to 8 kg. throughout the whole course of her pregnancy. Very little gain in weight occurs in the first three months. The average gain for the remainder should then be a maximum of 225 g. (½ lb.) per week. The incidence of toxæmia is found to be increased in those patients who gain more than 13 kg. Accordingly, he restricts the caloric intake to 1,800 calories, to be decreased or increased according to the patient's gain in weight. In Dieckmann's own series of 100 cases, however, the average gain in weight was found to be 9·5 kg.

Loughran in a series of 325 cases achieved very remarkable results through weight control. He restricted the gain in weight for a normal pregnant woman to 15 lb., the larger the woman the less the gain allowed. For the unfortunate obese, no gain was permitted. This he attained by a high protein high vitamin, low carbohydrate diet with adequate exercise and repeated check on the fluid balance. In this series, no patient developed toxæmia, the labours were of short duration—average 7·19 hours for a primipara and 5·15 hours for a multipara. There were very few dystocias and a low cæsarean rate.

Protein.—The development and growth of the fœtus and placenta together with the growth of the maternal tissues result in the storage of 200 g. to 400 g. of nitrogen which is equivalent to 1,250 g. to 2,500 g. of protein. This necessitates daily increase of 10 g. to 20 g. of protein in the maternal diet. The National Research Council of U.S.A. has recommended an intake of 8·5 g./day or 1·5 g./kg.

Diet studies cited above have shown that the protein intake of the great majority of pregnant women is well below this level, only 10 per cent in Burke's series, 13 per cent in William's and Fralin's and 19 per cent in Dieckmann's having an intake above 85 g.

Western workers recommend that 50 per cent of the total protein intake should be of animal origin, as the digestibility and biological value of animal protein is considered to be higher than that of vegetable protein. The studies of Patwardhan *et al.* (1949) do not support this. In a study of protein metabolism in 4 healthy adults they have recorded that the amount of nitrogen excreted in the urine per 24 hours was higher on diets containing a high proportion of animal protein than on predominantly vegetarian diets the fæcal nitrogen remained unaltered or showed insignificant variation. The nitrogen retention was higher with vegetable protein than with animal protein diet. The apparent digestibility of protein in the 2 diets was practically of the same order. In a further study (Patwardhan *et al.*, 1950) on 7 adults they confirmed their previous findings. They also found that the biological value and digestibility of vegetarian diet were not inferior to the vegetable animal protein mixture. There was, however, one difference—the body-weight changes were found to be in proportion to the positive nitrogen balances of subjects on an animal protein diet. But there was little change or even loss of weight when they were on a vegetarian diet though the nitrogen retention was higher.

Diet surveys in Coorg and Hyderabad have shown that the intake of animal protein is very low among our population due to poor economic conditions and social and religious objections.

An adequate intake of protein during pregnancy has been shown to improve the general well-being of the mother and her infant and her subsequent ability to nurse her baby.

Leverton and Macmillian (1946) conducted experiments on 33 pregnant women. They were divided into 3 sets of 11 each, one being given a supplement of 5 oz. of lean meat, one a supplement of B complex, and the third no supplement. The women who received meat showed consistently better hæmoglobin and red cell values at all times than the other two groups. These values were maintained after delivery. The meat groups showing a 20 per cent higher value than when supplementation was begun as against 7 per cent and 4 per cent for the two others. They also had less œdema and better success in lactation than the other groups.

The old view that a higher protein diet predisposed to toxæmia of pregnancy has given way to the opinion that the very opposite is true. Diet studies, however, have failed to give any positive proof.

Blair, Porter and Atkinson (1946) consider the 85 g. of protein per day recommended by the U.S. National Research Council the minimum and a safer figure is 100 g. to 120 g. They say that as increased protein loss is most commonly due to its excretion through the kidneys, the appearance of proteinæmia should be a signal to keep up plasma proteins, not to lower the intake.

Other workers have not found that dietary deficiency of protein causes toxæmia or that the protein intake of women who developed toxæmia was lower than those who did not (Williams and Fralin 1942; Hobson 1949; Dieckmann 1948). Smith has shown that under conditions of famine in Holland, the incidence of toxæmia fell from 3·2 per cent to 1·9 per cent.

The Vanderbilt Co-operative Study of Nutrition has found that women who later developed toxæmia had a lower intake of protein especially in the first and third trimesters. The serum proteins, however, showed no fall and prior to the recognition of toxæmia there was not a lowering but elevation of the total serum proteins. They did not support the view that pre-eclampsia and eclampsia are due to protein deficiency.

When fœtal development is concerned, the workers consider that protein intake is related to the length and weight of the infant and birth and the maturation of its epiphyseal centres (Burke *et al.*, 1948; Smith 1947). Sontag and Wimes (1947) studied 203 cases and found no unequivocal relationship. There was a suggestion of a relationship between weight, length and protein intake and they felt that very low levels have to be reached before any effect was demonstrable.

Tompkins and Wiehl 1954 *x*-rayed 300 babies born at term and found no significant relationship between maternal diet and maturation of epiphyseal centres.

There is a general agreement that a diet adequate in protein during pregnancy and after delivery makes for successful lactation.

Calcium.—The factors influencing calcium metabolism have been enumerated by Obermer 1946:—

1. Gastric acidity.
2. Potential acid-base balance of ingested food.
3. Acid-base balance of the body as a whole.
4. Acidity of urine.
5. Ca: P ratio in the diet.
6. Ingestion and digestion of fats.
7. Motility of intestinal tract.
8. Parathyroid function.

9. Thyroid function.
10. Ovarian, pituitary and other endocrines.
11. Vitamin D ingestion and synthesis.

In a series of controlled experiments he found that there was always a tendency for negative calcium balances in pregnant women, supplementing the diet with calcium alone or calciferol alone, did not produce positive balances and that calcium together with enormous doses of carciferol were required before a positive balance was obtained.

Newman (1953) studied the serum calcium (total and ionised) levels in 107 pregnant women and the effect of medical administration of calcium and vitamin D. He found ,that the average serum-calcium level was 8·5-11mg./100 mg. and that the administration of calcium with or without vitamin D did not raise the level; the level of ionised calcium being slightly depressed. He suggests that increased supply of calcium and vitamin D disturbs the normal parathyroid metabolism.

Medicinal administration of calcium is not advisable or desirable. An intake of 1 quart of milk a day ensures the necessary intake of calcium besides maintaining a satisfactory Ca : P ratio.

Iron.—Venure and Klopper (1951) have discussed the metabolism of iron and the changes it undergoes in the pregnant and puerperal woman. All iron ingested has to pass through the intermediary stage of serum iron before it is converted into tissue iron or into hæmoglobin. This serum iron is found to be bound with the P. globulin fraction of the serum proteins, the fraction of B. globulin not bound with iron being called the unsaturated iron-binding capacity. During the first 20 weeks of pregnancy, not much change occurs in iron metabolism. At that stage, the serum iron begins to fall till just before term when the lowest values are obtained (75 g.). The level rises again in the puerperium. The unsaturated and total iron-binding capacity rises from the 20th week and falls in the puerperium. The free protoporphyrin behaves in a similar manner.

In a latter study (1952) they found that these changes were considerably more pronounced in hypochronic anæmia, and have reported the effect of intravenous administration of iron. Along with clinical improvement and rise in hæmoglobin level, the serum-iron levels rose, and the iron binding capacity of the serum fell.

The work of Khan *et al.* (1951) also proves that it is in the latter half or latter third of pregnancy that the iron requirements are greater. Iron taged with radioactive isotope Fe 59 was fed in single doses ranging from 1·8 mg. to 120 mg. to 466 women at various stages of pregnancy and the uptake of iron determined. As dosage was increased from 8 mg. to 9 mg. to and beyond, the percentage of the total dose of iron found in the maternal red cells decreased while the amount of iron increased from the lowest to the highest dose. As gestation progressed, the uptake of iron increased to the extent that at 30 weeks, and over, three or more times as much iron was absorbed as before the 15th week. Before 20 weeks, multiparous women take up more iron than primiparæ, probably due to their lower iron stores. The increased uptake in both, in later pregnancy, masks any difference.

There is considerable difference of opinion regarding the physiological or pseudo-anæmic pregnancy. Mager and Milligan (1951) found that a gravida taking iron had a higher level of hæmoglobin, the effect being more pronounced, the longer the iron was taken, values as high as 88·5 per cent being attained. Since the authors report the same figure for their patients a year postpartum, it follows that the concept of pseudo-anæmia cannot be true.

Sikerise, Benstead and Theobald (1952) divided 72 patients at or before the 16th week of pregnancy whose initial Hb levels were not below 11 g. per cent into two matched groups. One group received ferrous sulphate 6 gr. tds. and the other a placebo. The mean Hb level of the first group at the 16th week was 90·8 per cent and remained at 90·6 per cent throughout pregnancy. On the other hand, the hæmoglobin in the control cases which averaged 88·8 per cent at 16 weeks, fell progressively to reach 76·6 per cent at 32nd week. Patient receiving iron did not, therefore, exhibit the pseudo-anæmia of pregnancy.

On the other hand, the studies of Lund (1951) and Dieckmann (1950) have shown that a fall in hæmoglobin levels occurs in spite of the routine administration of iron.

In a survey of the hæmoglobin levels in Coorg, Phave and Bopaiya (1942), it was found that the average Hb level of non-pregnant women was 10·13 g. per cent and the average in 150 pregnant women was 8·16 per cent, 35·3 per cent having a level below 7·7 g. per cent.

It is, therefore, recommended that all pregnant women should receive iron routinely throughout gestation, particularly after the 20th week of pregnancy.

VITAMINS

Vitamin A.—Investigation of serum vitamin A levels (Daeby *et al.*, 1948) have shown that there is a fall during successive stages of pregnancy, with a marked rise in the postpartum level. Carotene levels showed an increased relationship to vitamin A levels, distinct increments occurring from trimester to trimester with a fall in the postpartum period.

Warkany (1948) in his experiments on rats has shown that female rats raised on a diet poor in carotene and vitamin A and on a diet completely free of this vitamin had a low fertility and the young ones born did not survive. These showed various soft tissue anomalies—they were reduced in size, appeared œdematous and frequently had subcutaneous hæmorrhage. Some had open eyes due to defects of the lids. Anomalies of various ocular structures occurred, the most frequent being a retrolenticular fibrous mass in place of the vitreous body. Defective development of lungs and pleural cavities, diaphragmatic hermias, nonfunctioning kidneys, horseshoe kidneys and undescended testes were some of the other anomalies.

Such severe deficiency, however, is not likely to be found in human adults, and it is not known what effects may be seen in the human infant.

The anti-infective rôle of vitamin A in the prevention of puerperal sepsis was not supported by the work of Williams and Fralin who found no difference in the intakes of vitamin A and C in the diets of patients who had a febrile puerperium and those who were febrile.

The serum vitamin A level in those who had puerperal complications or fever in the Vanderbilt group, was found to be lower than the average; but as the level was always above 100 I.U./100 c.c. the signifiance of the observation is doubtful. A daily intake of 600 I.U. units is recommended in pregnancy.

Vitamin D is initially linked with calcium and phosphorous metabolism. Its importance in the prevention of osteomalacia, rickets in the child, and faulty dentition have been emphasized by Maxwell and Green Armytage and experimentally demonstrated in puppies by Mellanby. Not much has been added in recent nutrition studies apart from that studied under calcium.

Vitamin B_1 deficiency and beri beri is common in China and other parts of the world where highly polished rice or white flour is extensively used. Nixon and Gordon King found a very high incidence of preeclamptic toxæmia in these women.

Hyperemesis may produce deficiency of vitamin B_1 due to poor intake. The administration of large quantities of glucose by the intravenous route may aggravate the condition as vitamin B_1 is essential for carbohydrate metabolism.

The polyneurites of pregnancy (dry beri beri) which may manifest in later months of pregnancy is often due to a deficiency started by hyperemesis.

Vitamin B_2.—The Palestine workers (Bromberg, Bram and Braziashi, 1945) found deficiency of this vitamin to be very common—190 out of 900 pregnant women—the symptoms being glossitis, heartburn, cheilosis and angular stomatitis, vomiting in the second half of pregnancy was a feature of importance in these women. There was no difference in the incidence of toxæmia and other complications of pregnancy and labour. But the prematurity rate was higher and lactation poorer among these women.

Warkany (1948) bred female rats on riboflavin-deficient diets. These gave birth to young ones with congenital skeletal mal-formations, short bones, syndectylism, cleft palate, etc. He suggests that the development of the membranes requires a high riboflavin level. The same results were not, however, obtained when different species and strains were used.

Niacin.—During pregnancy the excretion of N. methyinicotinamide in the urine was found to rise and there was postpartum fall to pre-pregnant levels.

Sidall noted that the incidence of eclampsia is highest in the S.E. quadrant of U.S.A. which also has the highest incidence of pellagra. Both showed a similar seasonal variation—highest in late winter and early spring.

Hobson also has found that the diet of 17 toxæmia cases were good in other respects, but the nicotinic acid intake was low—8·3 mg. compared to 10·3 mg. of the 94 non-toxæmic.

The Vanderbilt nutrition study has found a low intake of this vitamin among pre-eclamptic and eclamptic patients associated with a decreased excretion of N. methylnicotinamide.

No definite causal effect has been claimed, but it is not unlikely that deficiency of one or all of the B complex group may be involved in the production of toxæmia.

Vitamin C.—Young (1946) has found a fall in the level of serum ascorbic acid, as pregnancy advanced and marked rise 6 to 12 months postpartum.

Similar values were obtained by Darby (1948) but he found that during the 6 weeks, postpartum examination, women who were lactating showed a further steep fall, whereas, in women who did not nurse their babies the level was restored to normal. Seasonal trends were also seen, the highest levels being obtained in summer and the lowest in winter.

Most pregnant women who manifest deficiency have latent deficiency in the non-pregnant stage and pregnancy is only an aggravating factor.

4. Study of the nutritional status of expectant mothers with particular reference to the incidence of toxæmias of pregnancy and anæmias in pregnancy in Northern Madhya Bharat under Drs. M. K. Lokre, B. K. Aikat and K. Sagriya at the G. R. Medical College, and K. R. Hospital, Gwalior.

Incidence of toxæmias of pregnancy and anæmias in pregnancy is variable in different parts of India. In the present study, out of 565 total deliveries, 26 are toxæmias, 20 are toxæmias associated with anæmia and 72 are anæmias. The clinical characteristics of the syndrome of toxæmia is alike to both the groups of toxæmias. The degree of anæmia shows no relation to the severity of toxæmia, but an anæmic woman is 4 to 5 times more liable to develop toxæmia than a pregnant woman with normal hæmoglobin level. Hæmatocrit values of toxæmias associated with anæmias show no special affinity for any group of anæmias. In 55 cases of anæmia where a complete hæmograme is done, the cell type is as follows: macrocytosis, with hyperchromia or occasional hypochromia in 17 cases, normocytosis with normochromia in 25 cases and microcytosis with hypo or occasional normochromia in 13 cases.

Data on nutritional survey are very small to draw any conclusion.

All cases of toxæmias and anæmias that were admitted in the Obstetstric Department in the months of July, August, September, 1955 are taken up for the present study. The cases are divided into three groups, (1) toxæmias with normal hæmoglobin level, (2) toxæmias associated with anæmia, and (3) anæmias.

Toxæmia

Hypertension.—Raised blood pressure is the main criterion for including a case in this group. A systolic pressure of 130 or above and or a diastolic pressure of 90 or above after the 24th week of pregnancy is taken as the border line. A case who maintains blood pressure at or above these levels for 24 hours or more is labelled as toxæmic. These cases are graded into 5 groups :—

Grade	I	systolic	pressure	130 and	diastolic	pressure	below ...	90
,,	II	,,	,,	140 and, or	,,	,,	...	90
,,	III	,,	,,	150 and, or	,,	,,	above ...	100
,,	IV	,,	,,	160 and above and, or diastolic pressure 101 and above				
,,	V	Any of the above groups associated with convulsions.						

Majority of the cases were unbooked and those that attended the antenatal clinic were seen after the 24th week of pregnancy, hence no attempt is made to divide these cases into pre-eclampsia and chronic hypertensive diseases, but in a few cases where a suggestive data of chronic hypertension was available is noted.

Protein-uria is assessed in term of 'trace', (cloudy),+,++and solid.

Œdema is noted.

Weight is recorded on admission.

Seasonal variation.—To ascertain the seasonal variations, the monthly record is shown separately.

LABORATORY INVESTIGATIONS.

Hæmatological examination.—Complete hæmograme.

Urine.—Routine examination including microscopic examination. Total plasma proteins and albumin globulin ratio; and serum calcium are done.

NUTRITION SURVEY.

Nutritional status.—Assessment of the nutritional status is done on the lines of 'Nutritional Rapid Survey' schedule recommended by the Nutritional Advisory Committee.

Per capita income.—Family members are recorded separately as adults and children below 14 years. Two children are taken equal to one adult. Total family income divided by the family members gives the per capita income.

Daily diet history.—Diet history is taken in detail in terms of atta, rice, dal, fats, oils, milk, eggs, fish, meat, green vegetables, other vegetables, fruits, the calorie intake and nutritional values are calculated according to the Health Bulletin No. 23.

Clinical examination.—This is done to find out the stigmata of the various vitamin deficiencies. Height and weight is recorded to compare it with standard weights against heights and age chart. But as the weights were taken during pregnancy, they could not be compared with the standard weights which are recorded on the non-pregnant women.

TOXÆMIA ASSOCIATED WITH ANÆMIA AND ANÆMIAS.

These groups are also investigated on the same lines as above. A case is called anæmic if the hæmoglobin is below 10 g. per cent. Hæmoglobin level of 14·8 g. per cent is taken as normal.

TOXÆMIAS.

Severity.—Tables II and III give the gradations of both the groups of toxæmias. Œdema of severe degree and proteinuria appear in a toxæmic patient when the blood pressure is raised to 150/100 mm. Hg. but a toxæmic case associated with anæmia shows trace to +proteinuria at 130/90 mm. Hg. and the œdema is directly proportional to the severity of anæmia. In both the groups after delivery or after the death of the fœtus in the utero, blood pressure dropped down to normal level, proteinuria disappeared and the œdema subsided.

TABLE I.

Incidence.

Month	Total deliveries	TOXÆMIA		ECLAMPSIA		TOXÆMIA ANÆMIA		ANÆMIA	
		Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent
July	172	13	7·0	2	1·1	6	3·4	27	15·6
August ...	182	4	2·0	1	0·5	7	3·7	33	18·1
September ...	211	5	2·3	1	0·4	7	3·3	12	5·6
Totals ...	565	22	3·8	4	0·7	20	3·5	72	12·7
Toxæmia naæmia		20	3·5	...	...			20	3·5
		42	7·3	4	0·7			92	16·2

A rise of blood pressure from 105/70 to 135/85 in a young girl is a definite danger signal. This fact is well illustrated in eclampsia No. 2. (Table II). A young girl, 23 years old, primigravid attended ante-natal clinic twice. At 16 weeks the pressure was 105/70, at 28 weeks the pressure was raised to 130/80. At 24 weeks she was admitted in a semicomatose condition with blood pressure 190/110, œdema+and proteinuria solid.

Age and parity.—Parity and age distribution for both the groups of toxæmias is alike. There is a preponderance of primigravida and nearly half the patients are in the age group of twenty years.

Normal blood pressure.—The anæmic pregnant woman tends to keep blood pressure at the same level as the non-anæmic pregnant woman. This fact is brought out by comparing the pressure of 72 anæmic patients with 217 controls from the ante-natal clinic.

Mortality.—There are 3 deaths in the present group of cases; one is due to eclampsia (Table II) and two are due to anæmia with hæmoglobin 2·9 g. and 4·1 g. per cent.

Hæmatological examination.—In 26 toxæmia cases the hæmoglobin level is between 10 g. and 13 g. per cent and the erythrocytes are normocytic normochromic. In 20 cases of toxæmia anæmia group, the incidence of erythrocyte variation is on the same line as the anæmia group (Table VI).

DISTRIBUTION OF TOXÆMIAS IN VARIOUS GRADES.

TABLE II.

Toxæmias with normal hæmoglobin levels.

	GRADE I B.P. 130/90			GRADE II B.P. 140/90			GRADE III B.P. 150/100			GRADE IV B.P.160/101			GRADE V CONVULSIONS WITH ANY GRADE		
	BP.	Œdema	Albumin	BP.	Œdema	Albumin	BP.	Œdema	Albumin	BP.	Œdema	Albumin	BP.	Œdema	Albumin
July 1955	...	...	...	140/72	...	...	150/80	Feet	Trace	150/110	...	...	190/110	+	+
	130/90	...	...	130/90	...	140/100	140/190	,,	...	140/110	+	+	1 fit soon after delivery		
	136/92	...	...	136/92	...	...	150/90	,,	+	160/70	(Twins) ...	...	190/110	anasarca	Solid
	...	...	...	140/90	...	...	140/100			132/112	+	+	Semicomatose.		
				140/90	...	...									
Cases 15	0	...	...		5			4				4		2	
August		...	...	148/90	Feet	++	150/100	...	...	150/120	...	...	90/60	anasarca	Solid
										160/100		Trace	Many fits before admission comatose, in shock *Expired 6 hours after admission*		
Cases 5	...	...	...		1			1			2			1	

Table II—(*Concld.*)

	Grade I B.P. 130/89			Grade II B.P. 140/90			Grade III B.P. 150/100			Grade IV B.P. 160/101			Grade V convulsions with any grade		
	BP.	Œdema	Albumin	BP.	Œdema	Albumin	BP.	Œdema	Albumin	BP.	Œdema	Albumin	BP.	Œdema	Albumin
Sept.	...	...	...	140/90	Feet	...	150/90	...	+	—	...	...	170/100	...	Tr.
				140/80	...	...	140/100	...	...				3 fits post-partum		
							140/100								
							(Twins pre-eclampsia 2 years ago. Grand Father died of hypertension.)								
Cases 6					2			3						1	

TABLE III.

Distribution of toxæmias associated with anæmias in various grades.

	GRADE I B.P. 130/89				GRADE II B.P. 140/90				GRADE III B.P. 150/100				GRADE IV B.P. 160/101			
	B.P.	Œdema	Albumin	Hæmo-globin	B.P.	Œdema	Albumin	Hæmo-globin	B.P.	Œdema	Albumin	Hæmo globin	B.P.	Œdema	Albumin	Hæmo-globin
July 1955	130/80	+++	Tr.	2·9/g mac. hyper.	130/90	...	...	8·5/g N.N.	150/100	+	++	8·2/g N.N. (8·2/9)	170/100	+++ gr.— (gr.	++ Casts Casts)	5·0/g- mac hyper
					140/70	++	+	5/g N.N.								
					134/90	...	...	5/g.								
Cases 6		1				3				1				1		
August	130/80	+++	+	4g mac. hyper.		*Nil*			154/100	+++	++	3·6 g. N.N.	160/100	...	...	9·4g N.N.
										Vesicularmole						
	130/70	...	...	7·1/g N.N.					150/100	...	...	7·5/g mic. hypo.				
	130/70	...	...	4·3/g mic. hypo.					150/80	+++	+	2·9g				
Cases 7		3				...				3				1		

TABLE III.—(*Concld.*).

Distribution of toxæmias associated with anæmias in various grades.

	GRADE I B.P. 130/89				GRADE II B.P. 140/90				GRADE III B.P. 150/100				GRADE IV B.P. 160/101			
	B.P.	Œdema	Albumin	Hæmo-globin	B.P.	Œdema	Albumin	Hæmo-globin	B.P.	Œdema	Albumin	Hæmo-globin	B.P.	Œdema	Albumin	Hæmo-globin
Sept.			...		140/90	...	...	6·8/g.		...			160/100	+++	+	2·6/g. mic. hypo.
													190/110	...	...	7·8/g- gravida ONE 28 weeks accid. hæmo
															Calcification	Pit Fossa.
													190/140	++	++	8·7/g.
													160/110	+	+	7·6/g. mic. hypo.
													**Twins*			
													120/100	...	Solid	7·5g.
														Accid hæmo in shock		
													160/80	...	+	7·1/g.
Cases 7		...				1				...				6		

* Scanty urine, 4½ ozs. in 24 hours. Therefore surgical induction of labour with Flatus tube. Spontaneous delivery after 10 hours. Babies alive. suppression of urine for

Table IV.

	Parity				Age		
	Primi-gravida	Primi-para	II para	Multi-para	14-20 years	21-25 years	26 yaers and above
Toxæmia 26 cases ...	12	5	2	7	12	4	10
Toxæmia anæmia 20 cases ...	9	2	5	4	10	3	7
...	21	7	7	11	22	7	17
Anæmia 72 cases ...	15	9	17	31	23	24	25

Table V.

Blood pressure in normal and anæmic pregnant women.

	Mean	Maximum	Minimum
217 pregnant women with nor. hae. ...	$\frac{115}{75}$ B.P.	$\frac{128}{85}$ B.P.	$\frac{90}{60}$ B.P.
72 pregnant women with anæmia ...	$\frac{112}{73}$ B.P.	$\frac{128}{85}$ B.P.	$\frac{90}{50}$ B.P.

Table VI.

Hæmatological analysis.

	Cases	Hæmoglobin per cent	20 per cent 2·9 g.	30 per cent 4·3 g.	40 per cent 5·7 g.	50 per cent 7·1 g.	60 per cent 8·5 g.	69 per cent 9·8 g.
	3	Mac. hyper	1	1	1	...	...	...
	4	Micro. hypo	1	1	...	...	2	...
	6	Nor. nor.	...	1	1	1	1	2
	7	Not known	1	...	2	2	...	2
Toxæmia anæmia	20		3	3	4	3	3	4
	12	Mac. hyper	1	...	7	2	1	1
	1	,, hypo	1	...	0	...	...	...
	1	,, nor	...	1	...	...	...	...
	16	Nor. nor	1	...	1	5	3	6
	3	,, hypo	...	...	...	2	...	1
	8	Mic. hypo	1	...	1	3	3	...
	1	,, nor.	...	...	...	...	...	1
	30	Not known	1	1	3	9	15	1
Anæmia	72		5	2	12	21	22	10

TABLE VII.

	Mean	Maximum	Minimum	Cases
Total plasma proteins				
Toxæmia	6·15 g.	7·1 g.	6·1 g.	18
Toxæmia anæmia ...	6·4 g.	7·2 g.	5·4 g.	11
Anæmia	6·13 g.	7·2 g.	4·5 g.	15
Serum calcium				
Toxæmia	9·5 mg.	11·4 mg.	8·4 mg.	18
Toxæmia anæmia ...	7·8 mg.	10·05 mg.	8·4 mg.	11
Anæmia	9·62 mg.	11·4 mg.	8·6 mg.	15

TABLE VIII.

Diet and Nutrition survey.

	Total calories	Protein, g.	Milk, oz.	Green vegetable, oz.	Other vegetables, oz.	Vitamin deficiencies
Toxæmia.						
Mean	1,871	54·8	9·8	3/4	4·8	...
Maximum	2,369	71·8	24·0	2	12	...
Minimum	1,362	37·0	0·0	0	1	...
Total cases 15						
Toxæmia anæmia						
Mean	1535.3	50·9	3·76	1	2·6	Conjunctivæ not clear 1 case. Xerosis 1 case. Bleeding gums 2 cases. Bleeding Spongy gums 1 case. Caries teeth 4 cases.
Maximum	2490.0	84·4	16·00	4	6·0	
Minimum	861·5	27·7	0·00	0	1·0	
Total cases 15						
Anaemia						
Mean	1,665	53.3	6.4	0.9	3.7	Bitots spots 1 case. Caries teeth 5 cases. Bleeding gums 4 cases. Kylonychia 3 cases. *Pigmentation extremeties and face 1 case.
Maximum	3,255	94.0	16.0	4.0	0.6	
Minimum	*944	32.0	0.0	0.0	0.0	
Total cases 43						

TABLE VIII—(*Concld.*)

Per capita income per month.

	Rs.10	Rs.20	Rs.30	Rs.40	Rs.50	Rs.60	Rs.70	Rs. 100
Toxæmia total cases	1	7	5	...	2	1	1	1
Toxæmia anæmia	3	5	3	...	2	2	...	...
Anæmia	12	15	7	2	1	1	...	2

5. Nutritional assessment of pregnant women under Dr. B. D. Patwardhan and Shri N. C. Datta at the Grant Medical College, Bombay.

Sixty one cases were selected at random from the pregnant women attending the antenatal department of the Bai Motli Bai Hospital. The majority of the cases belong to poor families with a monthly income of Rs. 20 per head living in a single room tenement in a marked overcrowding and insanitary conditions.

A complete record has been maintained of obstetric clinical and dietary history as per Nutritional Assessment Schedule for Pregnant women. A short summary of salient features is included in this report.

Obsteric history.—The 61 cases consisted of 12 primiparas and 49 multiparas. All the multiparas had normal previous deliveries. The number of deliveries are as follows :

Number of deliveries		Number of cases
Between 2 and 3	...	24
,, 4 and 6	...	19
More than 6	...	6
	Total	49

GENERAL.

Age, height and weight.

The distribution of cases in the different age groups are given below:—

Age in years		Number of cases
Below 20	...	3
20 - 25	...	27
26 - 30		28
- 35	...	3
	Total	61

Height and weight.—The height varied from 4′-4″ to 5′-3″. The weight varied from 80 lb. to 130 lb. It is significant that the multipara group on an average had low body-weight.

Eyes.—Occular deficiency manifestations were less frequent. Loss of lustre and Betot spot were seen in 10 cases; pigmentation of conjunctiva in 5; corneal xerosis and vascularisation in none.

Dark adaptation tests have not been done so far.

Mouth.—Oral hygiene was poor in almost all cases. Twenty seven cases normal form gum and 30 cases had normal teeth. Forty four cases had bleeding gums. Dental caries was present in 31, of whom 8 had at least 5 caries teeth.

Typical angular stomatitis was seen in 3 cases.

Hair.—There was no evidence of abnormal falling or thinning of the hair or loss of lustre in any of the cases.

Alimentary system.—Twenty-one subjects complained of slight anorexia. Most of the subjects had intestinal disorders, such as constipation and diarrhœa at times.

Liver and spleen.—Liver was palpable in 3 cases; spleen in none.

Nervous system.—Thirty-two cases had calf tenderness; paræsthesia was present in 28 cases.

Skin.—Loss of lustre of the skin was observed in 30 cases in the multipara group, of whom 4 had rough and dry skin.

Œdema.—Of the 61 cases, 26 cases had slight pitting œdema of the dependent parts, such as ankle and feet. Only 1 case was admitted for gross œdema. Four cases having no œdema during seventh month developed slight œdema during eighth month, detected during follow-up.

Investigation.—The blood pressure, in most of the cases, was within the normal range. The distribution of the cases in the different range of B.P. is shown below:—

B.P. in mm. of Hg.		Number of cases
100/65 and below	...	18
105/115 60-70	...	23
116-125/75-80	...	18
140/100	...	2

In the multipara group, the B. P. was slightly in the higher range of normal. The two cases with blood pressure had œdema and showed presence of albumin in urine.

(*b*) *Albumin in urine.*—Albumin in urine was detected only in five cases. Four cases had no albumin in urine during seventh month, showed albumin in urine during last month of pregnancy.

(*c*) *Hæmoglobin.*—Of the 61 cases, 16 were clinically normal, the hæmoglobin of the red blood cells varied from 10 to 13·1. Anæmia of varying degrees was present in 45 cases, of which 10 had marked anæmia. The hæmoglobin content in the anæmia group varied from 6·83 g. to 10·8 g. per cent.

(*d*) *Serum calcium.*—Serum calcium was determined in 21 of the 61 cases selected. The values of serum calcium varied from 7·9 to 10·8 g. per cent—values below 9 g. per cent was found in only 3 cases.

(*e*) *Serum proteins.*—Determination of serum proteins was carried out in 33 cases, of which 21 cases from the I.C.M.R. group. The remaining

12 cases were from pregnant women at full term, as a preliminary study and not included in the I.C.M.R. study. The results are given in Table I:–

TABLE I.

Serum protein in pregnancy.

Number of cases	Total protein	Albumin in g. per cent	Globulin	Euglobulin
21 (ICMR)				
Range	6·1–7·47	Range 2·29-4·1	Range 2·4-3·8	...
Average	6·68	3·50	Average 3·17	...
12 full term	6·87	3·47	3·44	0·81–1·37

There is fairly good agreement between the results of two series. A decrease in serum albumin and a rise in serum globulin during pregnancy is in agreement with the results reported by other workers. A rise in globulin due to an increase in euglobulin fraction as seen in the 12 cases at full term may be correlated with the increase in gamma globulin reported by Lewis *et al.* (*Ind. Jour. Med. Sci.*) 1955.

TABLE I*a*.

Serum protein in a few individual cases of pregnancy associated with œdema, anæmia and infection.

Number (ICMR)	Total protein	Albumin, g. per cent	Globulin	Remarks
52	6·86	2·29	3·51	T.B. Lungs V.D.-R.L. positive
48	6·11	3·01	3·10	anæmia and malnutrition.
53	6·11	2·91	3·2	Anæmia and odema
46	6·50	4·08	2·42	healthy.

It will be seen from the results of Table Ia that a significant decrease in albumin occurs in pregnancy in presence of anæmia, œdema and infection.

Termination of pregnancy.—Of the 61 cases selected, 10 cases delivered. Eight cases had normal course of pregnancy. No abnormality was detected during the course. Two cases with high blood pressure had œdema and albumin in the urine. They were admitted into the hospital and were treated in the usual lines. In one of them prolapse of the cord occurred during parturation. All others had full term of pregnancy and delivered normally.

Case history.

(*i*) *I.C.M.R. case No.* 18.—Primipara, age 18 years, weight 82 lb.

Diet.—Poor and deficient in animal protein. Milk and egg was absent from the diet. Fat intake was low.

Clinically.—showed obvious signs of anæmia and malnutrition, had slight œdema during last month and trace of albumin in urine.

Delivery normal.—There were intra-partum or post-partum, complications, except that the patient was exhausted.

(*ii*) *Case* 19 *and* 21.—Multipara, had 2 previous normal deliveries.

Diet.—The patients belong to poor families with a per capita income of Rs. 15/- per month. One purely vegetarian. Diet deficient in protein and fat. Milk, egg, and fruits absent from the diet.

Clinically.—Both were slightly anæmic and showed signs of deficiency of vitamin B. Both had high blood pressure (140/100), slight œdema and trace of albumin in the urine.

Delivery.—In case of case 19, prolapse of cord occured during parturation. Otherwise delivery and paurpeurium was normal.

Child.—Normal.

(*iii*) *Case Nos.* 2, 4, 6, 7, 14, 43, 12.—All these cases belong to slightly higher income group. Clinically 4 were normal. Average Hb of 4 was 11 per cent 3 had slight anæmia. Slight œdema of ankle was seen in 3. Only 1 had slight albumin in urine. Blood pressure was within normal limits.

Diet.—This was adequate. Egg, meat and fish were taken regularly. Intake of milk was not adequate. Rice and wheat form the main diet.

Delivery.—In all these cases delivery was normal at full term and normal puerperium during their stay at hospital. Child was normal.

(*f*) *Stool.*—Round worm ova was detected in many cases.

(*g*) Routine $\left.\begin{array}{c}\text{VDRL}\\ \text{R M T}\end{array}\right\}$ was done in all cases.

Chest screening was done in all.

Diet history.—A considerable amount of difficulty has to be encountered to get a reliable record of dietary history of the pregnant women due to ignorance and the lack of co-operation on the part of the subjects. By a process close questioning, it is impossible to get an idea of the actual intake and the various articles included in the diet. The research assistant has made it a point to visit the houses of the subjects and to take a record of the various articles of the food purchased, and whenever possible to weigh articles of food before cooking. Although the intake of diet is mostly poor and inadequate due to economical reasons, the house to house visit will have an educative value.

The diet of the pregnant women selected for the study was inadequate and unbalanced in many ways. The diet of the most of patients consisted mainly of rice, dhal (tour dhal), vegetables and occasionally meat and fish. All except 4 are accustomed to mixed diet.

Rice formed the main article of the diet in all, but No. 6, did not eat rice at all. In twenty-three of the 61 cases, rice formed the main diet with lesser quantity of wheat or jowar. Thirty-one others took rice with slightly increasing quantities of wheat or jowar.

Meat and fish.—These are taken once in a week in nominal quantitive, egg is absent from the diet.

The intake of *milk* varied from 2 to 4 oz. per family, and taken mainly with tea.

Furits.—These absent from the diet.

Onions are consumed in large amounts, and potatoes in fairly good amount. The vegetables: kǎkri green, leafy vegetables, bringal, gowar, dried peas, porwal and ladies finger are the most common vegetables eaten by poor classes; cabbage, cauliflower are costly and rarely taken.

Sugar.—The intake of sugar varied from 2 to 6 lb. per family of 4 to 5 members, depending on the income and number of children in the family.

Fat.—The fat intake is very low and consists mostly of ground-nut oil. Butter or ghee is not taken at all.

TABLE II.

Relationship of height and weight of the mother with the weight and length of the baby at birth.

Case number	Sex of baby	WEIGHT IN LB.		Height of mother in ft.- inch	Length of baby, inch
		Mother	Baby		
18	Male	82	4-80	4′ - 10″	18
Mother	Anæmic				
19	Male	110	5 lb. - 10 oz.	4′ - 10″	20
21	Male	90	6 ,, - 2 ,,	4′ - 10″	18
12	Female	93	6 ,, - 4 ,,	4′ - 9″	21
5	Female	110	6 ,, - 5 ,,	5′ - 1″	19
43	Female	105	5 ,, 0	4′ - 9″	19
14	Female	118	5 ,, - 14 ,,	5′ - 2″	21
4	Male	102	5 ,, - 0 ,,	5′ - 2″	18
2	Female	105	6 ,, - 2 ,,	5′ - 2″	19
7	Male	107	4 ,, - 9 ,,	4′ - 9″	21

TABLE II*a*

The correlation of the Hb content of mother's blood and cord blood.

Case number	Mother's Hb in g. per cent	Hb in g. per cent I.C.M.R. cord blood
18	6·83	16·1
6	9·2	15·4 I.C.M.R.
4	13·1	17·5
7	13·0	17·5

From the result of these four I.C.M.R. cases and ten others of pregnant women during delivery, it seems that the Hb content of cord blood of babies of anæmic mothers do not materially differ from those of the babies of normal mothers.

6. Assessment of nutritional status of expectant mothers with special reference to anæmias and toxæmias of pregnancy under Dr. Chunilal Mukherji at the Medical College, Calcutta.

All investigations were done according to the prescribed proforma. Up to date 200 antenatal cases have been studied. Hæmoglobin was estimated by Sahli hæmometer, serum proteins by Greenberg's and serum calcium by.....................method. Every attempt has been made to select these cases in the first half of gestation and so far 155 cases have been followed up once a month. Maximum period of follow-up so far has been 3 months. A summary of findings is given below:—

(1) *Age.*—15 to 32 years.

(2) *Religion.*—Hindu, 196; Christian 2; Mohammedan, 2.

(3) *Family income.*—Rs. 100/-, 68 cases; Rs. 100/- to Rs. 200/-, 68 cases. Rs. 200/- and above, 64 cases.

(4) *Primi gravida* 58 cases. Multi gravida.142 cases.

(5) *Duration of pregnancy.*—First trimester—42 cases. Second trimester—158 cases. Third trimester—0 cases.

(6) *Height.*—4′-7″ to 5′-3″.

(7) *Weight.*—75 lb. to 145 lb.

(8) *Diet.*—Vegetarian, 18 cases; mixed, 182 cases; No milk, 20 cases. Less than 8 oz. 146 cases; 8-16 oz, 32 cases. 16 oz. 2 cases.

(9) *Hæmoglobin.*—

	Cases
10 g. per cent and above	58
9-10g. per cent ,, ,,	42
8-9 g. per cent ,, ,,	62
7-8 g. per cent ,, ,,	38

(10) *Stool.*—

	Cases
Helminthic infection	16
Protozoal infection	34

(11) *Urine.*—No abnormal findings in any subject.

(12) *Serum calcium.*—

	Cases
9-11mg. per cent	162
11 mg. per cent above	36
Less than 9mg.per cent	*Nil*

(13) *Serum protein.*—

	Cases
4 to 5 g. per cent	50
5 to 6 g. per cent	146
6 to 7g. per cent	4

(14) *Blood pressure.*—

	Cases
Hypertension	20
Normal B.P.	170
Hypotension	10

(15) *Nutritional status.*—

Pallor 44 cases
Conjunctival xerosis 12 cases
Eye pigmentation 28 cases
Lachrymation 2 cases
Dryness of skin, pachyderma 38 cases
Night blindness 6 cases
Stomatitis 16 cases
Spongy and bleeding gum 52 cases
Fluorosis 8 cases
Caries teeth 36 cases
Œdema 6 cases

7. Inquiry on the estimation of 17-ketosteroids content of urine in toxæmia of pregnancy with special reference to ætiology and prognosis under Dr. S. Mitra at the Chittranjan Cancer Hospital, Calcutta.

Since the I.C.M.R. grant for the above inquiry in April 1955, we have studied 122 normal pregnancy cases against 50 cases of toxæmia. We have been using the chemical assay method as described by King (Microanalysis in Medical Biochemistry, 1951, 2nd Ed.) and further modified according to the British Medical Research Committee (*Lancet*, 261, p.585, 1951) method.

Technique.—Step A—Collection of 24-hour urine with copper sulphate as preservative.

Step B—Hydrolysis of conjugated 17- ketosteroids with concentrated hydrochloric acid.

Step C—Extraction with carbon tetrachloride.

Step D—Washing with water, sodium hydroxide, etc.

Step E—Colorimetric estimation by Zimmermann's reaction.

TABLE I.

Daily 17 ketosteroids output in normal pregnancy
(Total number of cases 122.)

Weeks of pregnancy	Number of cases	17-ketosteroids in mg./24 hours	Weeks of pregnancy	Number of cases	17-ketosteroids in mg./24 hours
Upto 30	38	10·64	31–32	13	7·21
					15·07
		13·24			27·48
					8·73
		12·75			6·82
					8·94
		6·99			4·90
					6·49
		8·74			20·60
					9·06
		1·42			4·18
					7·28
		7·50			5·54
		1·26	33–34	10	25·80

TABLE I—(*Contd.*)

Weeks of pregnancy	Number of cases	17-ketosteroids in mg./24 hours	Weeks of pregnancy	Number of cases	17-ketosteriods in mg./24 hours
		6·50			12·48
		11·37			4·29
		34·58			5·71
		7·80			4·21
		4·88			9·82
		7·33			13·89
		3·20			12·66
		26·75			11·60
		15·40			5·42
		20·75			
		9·24	35–36	15	23·61
		10·01			4·52
		15·08			5·82
		7·66			10·55
		13·75			27·45
		14·72			11·75
		12·53			13·58
		13·91			5·07
		2·95			10·00
		3·97			3·21
		11·98			6·66
		8·76			5·16
		6·37			8·97
		6·23			6·31
		6·56			4·82
		4·83			
		7·34			
		9·85			
		3·85			
		2·85			
39-40	23	6·42	39–40		
		14·68			19·30
		19·19			8·99
		14·11			10·32
		9·89			4·08
		7·68			17·98
		8·12			19·60
		7·69	Above 40	9	16·94
		4·32			9·64
		4·88			4·14
		11·39			14·28
		4·10			4·19
		7·46			2·07
		15·60			11·04
		22·12			10·37
		15·10			11·80
		12·05			

Table II.

Daily 17-ketosteroids output in toxæmia of pregnancy
(Total number of cases 50).

Weeks of pregnancy	Number of cases	17-ketosteroids in mg./ 24 hours	Weeks of pregnancy	Number of cases	17-ketosteroids in mg./ 24 hours
Up to 30	7	1·06	37–38	10	13·77
		2·41			13·04
		3·10			5·14
		6·02			9·19
		9·06			4·90
		7·58			3·71
		5·51			8·12
					11·09
31-32	7	11·07			8·22
		15·16			10·85
		5·42	39–40	12	2·16
		18·59			5·25
		16·20			5·62
		3·20			8·10
		2·46			7·61
33–34	3	29·16			2·46
		4·18			13·24
		7·84			9·92
					4·51
35–36	8	34·56			6·40
		3·22			34·77
		0·98			8·33
		10·62			
		6·42			
		8·32	above 40	3	21·46
		1·06			6·94
		26·06			7·70

Table III.

Average daily 17-ketosteroids excretion in normal pregnancy.
(Total number of cases 123).

Weeks of pregnancy	Number of cases	Average daily output of 17-ketosteroids
Up to 30	38	9·83
31–32	13	10·15
33–34	10	10·58
35–36	15	7·65
37–38	15	9·84
39–40	23	13·09
Above 40	9	9·38

From Table III and the Graph we find that in our 122 normal cases there is a single peak at the 39–40 weeks. The curve remaining almost steady up to 33–34 weeks, falls at 35–36 weeks to rise again at 39–40 weeks to reach the peak. It falls after the 40th week, in cases of prolonged pregnancy the average daily output being 10·26 mg. (Tampan, 9·51 mg.)

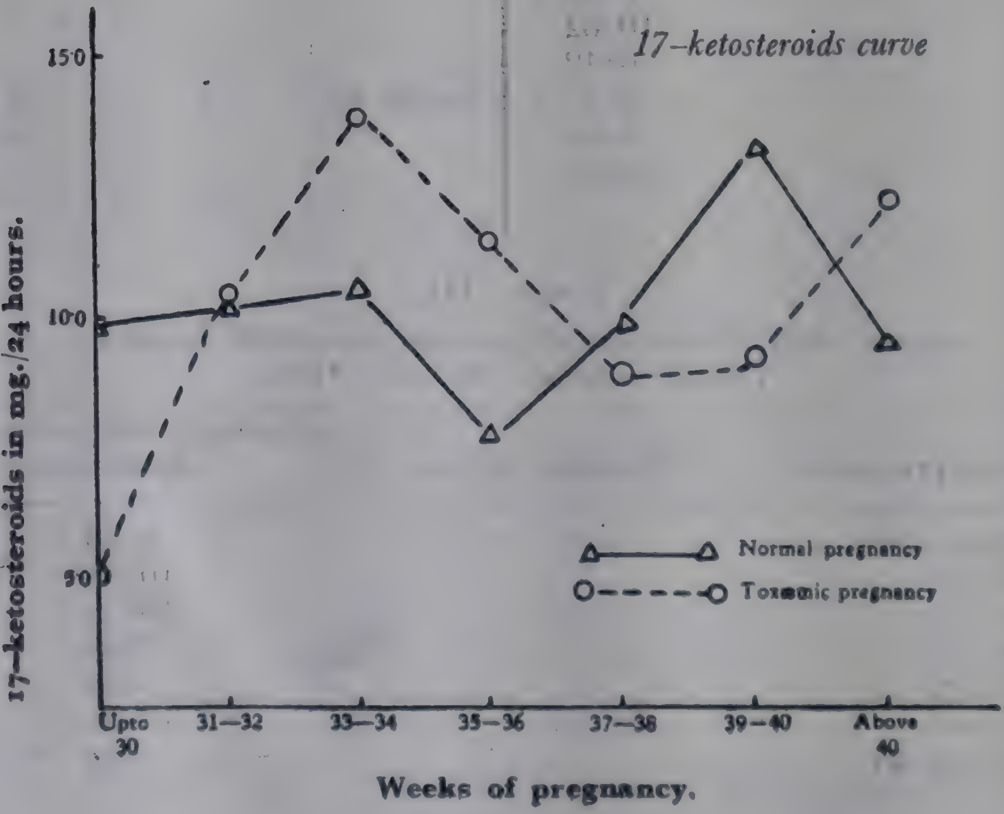
TABLE IV.

Average daily 17-*ketosteroids excretion in toxæmia of pregnancy.*
(Total number of cases 50).

Weeks of pregnancy	Number of cases	Average daily output of 17-ketosteroids
Up to 30	7	4·96
31–32	7	10·30
33–34	3	13·73
35–36	8	11·40
37–38	10	8·80
39–40	12	9·03
Above 40	3	12·03

From Table IV and the Graph we find that in our 50 toxæmia of pregnancy cases, a sharp rise, much above the normal, at 33–34 weeks with a sharp fall till 37–38 weeks, whereafter the level reaches below normal and remains more or less constant till term; it again rises after 40th week and reaches as point above the normal. At 39–40 weeks the toxæmia

GRAPH.



curve remains far below the normal curve. The average daily output in toxæmia of pregnancy is 9·44 mg. (Tampan, 10·07 mg.).

DISCUSSION.

Taking Tampan's figure of a daily output of 6·4784 mg. as the mean daily output in normal non-pregnant Indian women we find that there is a definite increase in level in both normal pregnancy as well as in toxæmia of pregnancy. But no significant difference of values between the normal and toxæmic pregnancies was observed. In fact, we find that the average output in toxæmia cases is slightly less than that in normal cases.

Tampan and Ramaswamy (*Jour. Obst. Gynæc.*, India, **4**, 323, 1955) in a series of 59 normal pregnancy cases found the values within normal range although the mean values were increased. In toxæmia (50 cases) they have found the values still more increased.

Venning (*Endocrinol.*, **39**, p. 209 1946) by biossay methods, have found an initial slight rise followed by a fall and a subsequent sharp rise with a final drop in the excretion of urinary corticoid during normal pregnancy. Pincus *et al.* (Hormones *i*, N.Y., 1948) have observed that when progestrone secretion increases enormously during pregnancy, the urinary 17-ketosteroids remain relatively constant.

Curstensen found urinary corticoids in mild and severe preeclampsia lower than in normal pregnancy but high in hypertensive toxæmia of pregnancy where as Devis *et al.* have found high values in toxæmia and referred to Seyle (Text of Endocrinology, 1950) who suggested that eclampsia is a disease of adaptation because there is a similarity between the symptoms of eclampsia and those caused by DCA intoxication.

According to the suggestion of Dieckman (Toxæmia of Pregnancy, 2nd Ed., 1952) that sodium might be the toxin of toxæmia, it is expected that there should be an increase in the secretion of mineralocorticoids to produce the sodium intoxication and consequently the syndrome of toxæmia. Thus one is tempted to correlate the adrenal cortex with the ætiology of toxæmia of pregnancy.

We have not yet been able to come to any significant conclusion from the results of the small number of cases we have assayed during the last six months, to accept Seyles' suggestion that eclampsia is a disease of adaptation. Further investigation might bring some light on the above hypothesis.

8. Study of Rh factor in pregnant mothers and its importance in toxæmia of pregnancy and hæmolytic disease of the new born under Dr. Y. G. Bhide at the J. J. Group of Hospitals, Bombay.

Persons on whom Rh tests are carried out are divisible into four categories:—

(*i*) Females that have not been pregnant.
(*ii*) Females that are pregnant.
(*iii*) Females that have been pregnant.
(*iv*) Males.

Nurses of J. J. Hospital have volunteered for group one.

The antenatal cases of Motlibai Hospital form the 2nd group.

The females of Beggar Home, Chembur, have been included in 3rd group.

Blood bank doners of J. J. Hospital, form the 4th group.

First group gives the idea of frequency of Rh negative females in a population that is likely to be effected as tested by anti–D sera.

Second group helps us to sort out the likely current cases of sensitization.

The third group has been studied to look out for past sensitization.

The fourth group has been formed with a view to meet the demand of Rh negative blood for transfusion in Rh negative cases.

METHODS FOR RH TESTING.

1. Tube-test. 2. Diamond's rapid slide test.

Diamond's method.—An oval about 1″ × 2/3″ is marked on an ordinary glass-slide using glass marking pencil. A drop of anti-Rh serum and two or three drops of oxalated whole blood are mixed within the oval and spread out so as to cover the area of 1″ × 2/3″. The slide in now placed on a slightly warmed surface illuminated from below and rocked. If a potent anti-D serum is used, massive agglutination occurs within thirty seconds.

Modification of diamond's technique only as far as getting the warmed surface is concerned, other things being same.—To suit the local conditions, as the box viewer was not available, the following procedure was adopted:—

1. Slide was placed on the electrically operated microscopic lamp used as source of light. When testing on one slide is being done, the second slide is kept up for warming. The process of testing becomes continuous one without loss of time to get a warm slide. The warmth is judged by the palm of hand. The idea is to carry out the test at 37°C. The slide maintains the temperature for one minute during which time the reading is taken. The idea is conceived from the observation that the microscopic mirror is used in reading the Rh test. So, also the source of light can be used to get a warm slide.

2. Another method to get a warm slide that has been practised is the use of spirit lamp flame. The slide is passed through the flame till the heat can be tolerated by the palm of hand. Two drops of blood are secured and placed in a drop of serum on the warmed slide. The mixture is affected by the needle used for pricking the finger and the test is read in 30 seconds to 1 minute before blood is allowed to dry. Positive and negative controls have been kept. The tests were confirmed by tube-test. The test is simplified to work at places where facilities for incubation and electricity are not available. The results are reliable to the same extent when carried out by Diamond's technique. The results of the tests are given in Tables I to V.

TABLE I.

Rh testing by anti-D sera.

Would-be mothers.

Total tested	Rh positive	Rh negative	Rh negative percentage
100	95	5	5

TABLE II.

Antenatal cases.

Total tested	Rh positive	Rh negative	Rh negative percentage
510	503	7	1·37

TABLE III.

Pregnant females.

Total tested	Rh positive	Rh negative	Rh negative percentage
150	149	1	0·6

TABLE IV.

Blood bank donors.

Total tested	Rh positive	Rh negative	Rh negative percentage
96	93	3	3·2

TABLE V.

Total.

Total	Rh positive	Rh negative	Rh negative percentage
856	840	16	2

COMMENTS ON TABLES.

Group 1.—Out of 100 cases tested 5 were Rh negative and none of them was sensitized.

Group 2.—The number of tests that have been carried out is small. Out of 510 cases tested, 7 were Rh negative. This gives us the Rh negative percentage of 1·37 which is lower than those of English people.

Group III.—One out of 150 cases is Rh negative. This is a beggar class of women. In American negroes 0·8 per cent are Rh negative, while in Chinese 1 : 150 is Rh negative.

Group IV. Out of 96 donors tested, 3 were Rh negative, the Rh negative percentage being 3·2.

If the figures from all the groups are added the percentage for Rh negative persons comes to 2 per cent as compared with 15 per cent of English population.

Experimental work.—It was decided to prepare Coomb's serum by immunizing the rabbit by Proom's method. The animal became immunized and a serum with a titre of 1:512 was obtained. This serum is being used for all the tests that are being done.

An attempt at the production of anti-Rh sera by sensitizing a human volunteer is in progress. Waller's technique of giving 2 c.c. injections at six weekly intervals is being followed, as in 6 injections out of 10 persons become sensitized against Wiener's technique in which he has injected 2 c.c. to 4 c.c. at an interval of 3 to 4 months and has got 2/3rds of volunteers immunized after 5 injections. The Diamond's technique is not being used because by his technique only 50 per cent of persons became immunized. Advantage is that the time interval is only 2½ months but the total number of injections is great, that is ten.

IMPORTANCE OF Rh FACTOR IN TOXÆMIAS OF PREGNANCY.

The problem can be approached from two angles. One is to find out cases of toxæmia of pregnancy and later on to find out the number that is Rh negative and has become sensitized. The second is to find out all Rh negative cases and to find out the percentage that shows toxic manifestations.

The blood pressure of and above 140 mm. of mercury is taken as a sign of toxæmia of pregnancy. Out of 937 cases examined 8 showed the blood pressure of 140 and above and out of these none were Rh negative. In none of these cases albumin was found in urine. The study includes cases from group 2.

So far as the study of above cases goes, it is concluded that Rh factor has no relation to toxæmia of pregnancy.

IMPORTANCE OF Rh IN HÆMOLYTIC DISEASES OF NEW BORN.

The sensitization is the production of the anti-bodies to a factor which is absent from the sensitized persons' erythrocytes. The presence of these antibodies in our series has not had any detrimental effect on the mother except for the fact that she is likely to get a hæmolytic reaction whenever she is transfused by the erythrocytes containing corresponding antigen. These results are in agreement with those of Mournt, Mollison and Race.

In order to detect cases of Rh sensitization the following methods were adopted:—

(1) Indirect Coombs test was performed on 232 bloods out of which 3 were positive.

(2) Coomb's test was performed on 16 Rh negative bloods and the test was positive in three cases showing that these three cases were sensitized. Two of the 3 have not delivered and the effect of the antibodies on fœtus is being followed up. A short case report in respect of these three cases is given below.

(3) 190 fœtal bloods were tested with direct Coomb's test but none of them was positive.

The above observations suggect that the best way to find out a case of Rh sensitization is to perform an indirect Coomb's test on Rh negative bloods of females that are carrying or have carried. Sensitization has been noted even in so short a time as one week, of course in a case in which a blood transfusion was effected.

Case reports:—

Case 1.—Mrs. Y.B.C., aged 35, 5 para in the 7th month, attended the antenatal clinic at Motlibai Hospital on 7-9-55.

Obstetric history.—1st child aged twelve, living healthy. 2nd child died of pneumonia at the age of one year. 3rd child died of jaundice at the age of one year. 4th child living and is 6 years old.

She gives hsitory that she has been in normal health in all these pregnancies. She gives no history of blood transfusion. At present she complains of slight weakness. On physical examination nothing abnormal is detected except a lower blood pressure of 105/65 mm. of mercury.

The following investigations were carried out:—

R.b.c.	Total	3·5 million
W.b.c.	,,	7,400 per cm.
Hb.	,,	11·6 g.

Blood group O ccdee
Direct Coomb's test—Negative.
Indirect Coomb's test—Positive.

Serum antibody titre as tested with husband's cells

in saline	1:0	1:0
	(7-9-55)	(25-10-55)
in albumin	1:512	1:1024

Serum antibody titre as tested with anti-A and anti-B.

Anti-A	7-9-55	25-10-55
	1:256	1:256
Anti-B	1:128	1:128

Husband's blood group is 0 and showed clumping as tested with anti-C.D.E.

The blood of children is not available.

The patient delivered 4 F.T.N.D. This suggests that the titre of antibody must not have been high so as to produce fœtal damage. The other possibility is that the husband must be heterozygous, i.e., Dd.

Baby is more liable to suffer from erythroblastosis fœtalis as result of hyperimmune type of antibody in the titre of 1:512. c. present in the serum.

The rise in titre a month after suggests that she may be harbouring an Rh positive fœtus.

The case is being followed up.

Case 2.—Mrs. M.A.M., 2nd para, has been spotted out in the routine Rh testing of antenatal cases. Mrs. M.A.M., aged 22, para 2, is in the 8th month of pregnancy.

Obstetric history.—1st child is 5 years of age and is in good health.

Baby gives no history of transfusion or injection of sera. She enjoyed normal health in a previous pregnancy. At present she does not complain of any abnormal trouble. On physical examination nothing abnormal is detected. The following investigations are done on her.

Blood group O, cc.dee
Antibody Titre.

In albumin	1:1024
Saline	1:0

Titre of anti-A is 1:32
Titre of anti-B is 1: 8
R.b.c. 4·9 million per cm.
Hb. 12·5g per 100 c.c.

Examination of husbands' blood:

Blood group.—AB1, his cells were clumped with anti C.D.E. The child's blood is not available for examination.

This patient is only in second para and harbours the immune type of antibody to the titre of 1: 1024, suggests that she may be carrying an Rh positive fœtus and that it is also likely that the husband is homozygous DD. Further progress is being studied.

Case 3.—This case was referred from B.J. Hospital, for sick children.

Patient Mrs. R.J.S. gives obstetric history as follows:

1st child	F.T.N.D.
2nd ,,	abortion 1½ months
3rd ,,	F.T.N.D.
4th ,,	,,
5th ,,	Is living, aged 11 months. It had jaundice and fever 3 hours after delivery.

It also shows mental retardation.

She gives no history of having received any blood transfusions. She gives no history of having had any toxic manifestation as a result of pregnancies.

Blood group—'A', cde.

Anti-body titre.

In saline	1:0
In albumin	1:1024.

The anti-B titre is only 1:64.

Examination of husband's blood.—Blood group, AB1. His cells show clumping with anti C.D.E. Blood grouping of children could not be done. This patient has four living children. This suggests that the husband may be heterozygous. The high titre of protein antibodies suggest that the patient is reacting slowly through her previous pregnancies. The titre is supposed to remain up even for years.

The complete report of these three cases will be presented when two cases have delivered. Husbands of case Nos. 1 and 3 appear to be heterozygous Dd, while husband of case 2 appears to be homozygous DD. This is from the number of living children in case 1 and 3 and pretty high titre of albumin antibody of case No. 2 even as early as second pregnancy.

9. Study of Rh factor in pregnant mothers in Indian States and its importance, if any, in toxæmias, still-births, neo-natal deaths and hæmorrhagic diseases of the new-born under the Principal, Christian Medical College, Ludhiana.

One hundred specimens of blood from antenatal cases and patients in the Maternity Block were tested for ABO and Rh (D)(d) groups. Out of one hundred women tested, eleven were found to be Rh negative. None of these eleven women had Rh antibodies in her serum. The percentage of women with a bad obstetric history was substantially the same in both the Rh positive and negative groups.

Procedure:—Collection of specimens.—Samples of venous blood were collected in plain tubes as for Kahn testing. A special proforma was completed by the house-doctor concerned for each woman giving her name and age, her husband's name, full obstetric history and relevant medical history (malaria, jaundice, previous transfusions). The bottom half of the form was returned to the ward or clinic giving the patient's ABO and Rh group, and, in the case of Rh negative women, the result of the test for Rh antibodies. A small card in English and Hindi with the result of the blood test was given to every woman grouped, with instructions to keep it carefully.

Laboratory procedure.—The sera were separated from the clots. The latter were washed with normal saline to obtain cells. These cells were washed three times with normal saline in the usual way. ABO grouping was carried out in the usual way with sera obtained from the Blood Bank Organization, Pusa Road, New Delhi.

Rh grouping was carried out with cells in 25 per cent ox albumen (Armour Laboratories, London) and incomplete anti-D dried serum (from the Lister Institute, London). Grouping was carried out in small precipitin tubes which were incubated at 37°C. for two hours. The results were then read, agglutination being looked for both with the naked eye and microscopically.

Agglutination indicated that the subject was Rh positive (D).

No agglutination indicated that the subject was Rh negative (d).

Test for Rh antibody in patient's serum.—The sera of Rh negative women were incubated with the red cells of a known O Rh positive individual at 37°C. for two hours, both in saline and in ox-albumen suspension. In the eleven cases examined no Rh antibodies were detected. The indirect Coomb's test was carried out in two cases where the history was suggestive but was negative in both cases.

Results.—

ABO grouping in all hundred women.

O 37 A 16 B 39 AB 8.

Rh positive (D): 89 Rh negative (d): 11

Analysis of Rh positive women.—

Primiparæ, attending during their first pregnancy ...	10
Normal multiparæ (no history of any abortions, still-births or neonatal deaths)	12
Affected multiparæ (with a history of at least one abortion)	67
Total ...	89

Affected multiparæ.—

History of neonatal deaths ...	3	(including one case of toxæmia).
History of abortions only ...	55	
History of still-births	11	(one with toxæmia and eclampsia).
History of three childrens' deaths at six months of age with jaundice...	1	case.

The incidence of bad obstetric histories in these Rh positive women is surprisingly high (75·3 per cent). It is probable that the house-doctors and midwives selected the patients with the worst histories for Rh grouping in spite of the laboratory workers' requests for specimens from all types of patients. However, these cases served as a useful control on the Rh negative women.

Rh negative women.—Eight of the 11 women had a bad obstetric history. Details are given in the Table.

It will be seen that eight out of these eleven Rh negative women had a bad obstetric history (72·7 per cent). This proportion is not significantly different from the Rh positive group but 11 is too small a number to dogmatize over. None of the women gave a definite history suggestive of Rh immunization; i.e. first one or two pregnancies normal, then children born progressively more anæmic and then jaundiced, culminating in premature births of jaundiced still-born infants or even macerated or œdematous fœtuses. In all the eight cases the abortions, still-births, etc., were mixed with normal deliveries in a purely haphazard order not typical of Rh immunization. For this reason, the workers were not very surprised that no antibodies were found.

TABLE.

Rh negative cases.

Initial	Age	Obstetric history	ABO group	Antibodies
S.K.	22	Primip. 2/12	AB	None detected
J.K.	16	Primip. at term	A	,, ,,
M.D.	20	1st child died in 24 hours after FTND	A	,, ,,
L.K.	23	Multi. *ii* 1st pregn.; abort. at 3/12	O	,, ,,
R.K.	27	Mult. 2 FTND, 1 abort. 3 PD, last(SB and breech) ...	A	,, ,,
M.K.	20	Mult. *ii*. 1st fœtus macerated with big meningocœle ...	B	,, ,,
L.	22	Mult. *iii*. 3 FTND ...	B	,, ,,
G.K.	40	Mult. *vi*. 4 FTND 5th SB, 6th SB (mother had eclampsia in last preg.)	O	,, ,,
K.K.		Mult. *iii*. 2 aborts. at 3/12 ...	O	,, ,,
M.	21	Mult. *ii*. 1st preg. 3½ months abort.	B	,, ,,
R.	25	FTND died at 2½/12 old 2/12 abort. 3rd preg.	B	,, ,,

Abbreviations. Primipip. = primipara; Multip. = multipara; Preg. = pregnancy; Abort. = abortion; /12 = Number of months; FTND = Full term normal delivery; SB = still-birth.

10. Inquiry on the birth-weight of babies of mothers from different income groups under Dr. S. T. Achar at the Medical College, Madras.

The birth weight of babies born in the following institutions was noted soon after birth; the weighing balance in each of these places was checked up periodically with the standard weighing machine in the department and the deviation from the standard weighing machine noted and allowed for in calculation.

	Babies
Government Women and Children Hospital, Madras ...	2,686
Gujarati Sahayaka Maternity Home	112
Lady Willingdon Nursing Home	19
Total ...	2,817

The economic status of the parents has been assessed by the income of the family. The diet of the mother in the antenatal period was also recorded. The birth weight recorded in these 2,817 babies is tabulated below:—

Statement showing the average weights of babies and also the income of parents.

Classification according to weight in oz.	AT WOMEN AND CHILDREN HOSPITAL, MADRAS				AT GUJARATI SAHAYAKA MATERNITY HOME			AT LADY WILLINGDON NURSING HOME		
	Number of cases	Per cent of total	Average weight in oz.	Average income, Rs.	Number of cases	Average weight in oz.	Average income, Rs.	Number of cases	Average weight in oz.	Average income, Rs.
11-20	1	0·04	20·0	40·0	...	...	...	...	...	...
21-30	3	0·11	26·6	46·6	...	...	...	...	...	...
31-40	12	0·45	36·2	33·8	...	...	...	...	...	...
41-50	15	0·56	47·2	41·3	...	...	...	...	...	...
51-60	54	2·01	54·2	44·4	2	54·0	153·0	...	...	...
61-70	94	3·50	66·0	48·3	2	66·5	97·5	...	...	...
71-80	288	10·72	76·3	48·2	3	76·0	160·0	...	...	...
81-90	440	16·38	89·6	54·5	14	86·5	222·42	1	83·0	above 500
91-100	769	28·63	95·8	54·0	25	97·4	206·5	1	91·0	„
101-110	486	18·09	105·8	69·2	32	104·9	205·6	7	105·7	„
111-120	366	13·62	116·2	66·6	22	116·3	219·5	6	114·6	„
121-130	113	4·21	125·2	82·5	6	124·3	371·0	...	...	„
131-140	40	1·49	131·2	126·3	6	135·6	236·6	1	133·0	„
141-150	1	0·04	148·0	174·0	...	...	...	2	146·5	„
151-160	4	0·15	158·0	357·5	...	...	...	1	156·0	„
TOTALS	2686				112			19		

It will be seen from this:—

(1) that the birth weight of majority of the babies is between 75 and 125 ounces.

(2) that the birth weight increases steeply in the income group of Rs. 130 above and.

Various other aspects of birth weight in relation to diet of the mother are being tabulated separately and will be subjected to analysis later on.

11. Studies on physical development of babies and dietetic consumption of mothers in prenatal periods under Dr. (Mrs.) P. Verma, Medical Superintendent, Rafat Maternity Home, Rampur.

The work on the subject was started on the 1st of May, 1955. To carry on the work, three types of sechedules were prepared in addition to the ordinary Red Cross antenatal cards being already used in this place. These antenatal cards help to give the other causes or setbacks in the antenatal periods that the mothers may have had and which might have affected the development of the baby in addition to the mother's diet in the period.

The schedules were as follows:—

1. *Schedule A.*—For judging the nutritional status of the mother. This schedule is filled up when the first contact with the mother is made during her antenatal period.

2. *Schedule B.*—For noting the average weekly dietetic intake of the mother in the prenatal period. The mothers after the first contact are asked to bring in their daily diet dairy—from which weekly averags are calculated and put in the schedule and any gross dietetic imbalance is noted in the remarks column.

3. *Birth-weight schedule C.*—This schedule is filled in at the birth of the baby. It also gives a sort of summary of the parent's economic condition and the components of the mother's diet during her antenatal periods.

The work is being carried out in the urban area and with a mixed type of population which mainly consists of low middle class families. The deliveries are taken up in the maternity home and we have domicilliary midwifery service as well. The cases have also been followed in the domicilliary area and the maternity home. The weight and length of the babies is taken when the cord ligatured and the baby has had the first bath, i.e. almost within the first hour of delivery. The weight and length of all the newborns (those in the domicilliary area) are taken within the first 12 hours of the birth and the data filled in. In the domicilliary area, however, the midwives conducting the cases do take the weight and length of the baby after the baby's first bath and before the cord dressing, etc. is done.

The weighing machine being used in the inquiry is U.S.A. made (Detecto beam type) and the weights have been tested and the zero of the machine is tested before the weight of the baby is taken.

Altogether 175 new-borns were taken up in the inquiry from 1-5-55 to 9-8-55.

The nutritional assessment of the mother in prenatal period who delivered in the first few weeks of the start of scheme was only based on their antenatal cards which had been previously filled.

The mothers bring their dietetic consumption diaries and one has to depend on what they tell. There is no method, however, to be sure that they are noting regularly and not omitting any thing. No dietetic diary could be kept for the mothers who had delivered in May. Their diet consumption was taken in rough usual diet that they told they had been taking.

Any results on the basis of these few cases cannot be drawn but it would be possible to form some idea of the whole thing in a few months more and also it would be possible to get proper figures in the cases that have been contacted now in early prenatal stages.

FILARIASIS.

1. Inquiry on the control of *W. malayi* filariasis in Travancore-Cochin, under the Director, Malaria Institute of India, Delhi.

While some knowledge has been gained in the use of diethyl-carbamazine against *W. bancrofti* in India and in other countries, the only report available of such studies against *W. malayi* is that of Wilson (1951). There was, therefore, an urgent need to carry out studies on the use of this drug against *W. malayi* in India. Detailed studies were planned on (*a*) the effect of the drug in persons with *Mf. malayi*, (*b*) the possibility of alleviation or elimination of reactions following diethyl-carbamazine therapy by administration of some antihistaminic, (*e*) to determine a dose regime suitable for mass therapy, and (*d*) to study the extent of control if any that could be established in a community endemic with *Mf. malayi*.

Nine groups, each of ten individuals with *Mf. malayi* infection were selected. Three groups were placed on hetrazan, one on 2 mg., one of 3 m.g.m., three others on hetrazan and one in 4 mg./kg./day for five days. Likewise, three groups were given banocide and the remaining three received banocide histantin in similar dose schedules. The three groups receiving hetrazan were given calcium gluconate 20 grains a day during the three preceding days. In all cases prior to therapy, and in as many as possible on the day following and 25 and 110 days thereafter, routine blood studies (total and differential w.b.c., total r.b.c. counts, hæmoglobin percentage and microfilarial counts) were carried out. In addition, fæces examination (before therapy and a month after therapy) was also made.

Results obtained.—These can be grouped as (*a*) effect on microfilariæ and (*b*) reactions noted (immediate or delayed).

Effect on microfilariæ.—A marked drop in the number of microfilariæ was noted in all cases in all groups, as noted from the blood examination on the day following completion of therapy in the different dose-schedules. At present all groups have been followed up to 25 days after completion of therapy, while only some of the groups have been followed up to 110 days after therapy. The analysis will be completed after all groups have been studied up to 110 days after therapy before choosing a dose schedule suitable for mass therapy.

Reactions.—In every case some or the other reaction, such as nausea, vomiting, anorexia, headache, body pains, fever or abdominal pains was noted. These reactions set in from 12 to 24 hours after administration of the first dose of drug and continued for two to three days. Temperature records (about 10 a.m. daily) showed that usually fever reached about 101°F. to 102° F. by about the second or third day of therapy and subsequently defervesced to become normal by nearly the fifth day, even though the drug was administered on the fourth and fifth days. There appeared to be no cumulative effect of the drug as there was no exacerbation of reactions with continued administration of the drug. In no instance it was necessary to interrupt therapy due to the onset of such reactions which passed off.

The drugs used in these studies were obtained through the courtesy of M/s. Lederle Laboratories (India), Bombay (hetrazan), of M/s. Burroughs Wellcome Ltd, (India) Bombay (banocide and banocide-histantin) of M/s. May & Baker (India) Ltd. (anthisan and phenergan).

No definite opinion can be given at this stage regarding the utility of anti-histaminic in the relief of rections due to therapy.

Hæmatological studies showed a characteristic feature and that was marked eosinophilia immediately after therapy. It continued for over a month in majority of the cases. Further observations are being made.

A number of individuals in each group passed live round worms during therapy producing a subsequent sense of well being.

VIRUS DISEASES

1. VIRUS RESEARCH CENTRE, POONA.

The Virus Research Centre was established in 1952 by the Indian Council of Medical Research, with assistance from the Rockefeller Foundation and the Government of Bombay. It is presently maintained jointly by The Indian Council of Medical Research and the Rockefeller Foundation.

The third full year of its establishment marked the achievement of a truly All-India rôle of the Virus Research Centre with field investigations, collections and laboratory virological study of human disease problems from Calcutta to Bombay and Delhi to Madras. In addition to interest in the arthropod-borne neurotropic virus infections, which loom larger as public health problems as defined by these investigations, work was continued with enteric viruses as a possible cause of Jamshedpur Fever. the encephalitis in children which was recognized as an epidemic problem last year (Khan, 1954) over much of north India for the first time.

Capacity for dealing with virus problems over a great geographical area has developed apace with installation of new equipment, expansion and maximum utilization of space and enlargement, training and experience of the technical and professional staff. Emphasis has been placed on developing laboratory facilities to support an increasing amount of field investigation. With maturation of these facilities Department of Virology with sections of Animal Virology, Tissue Culture and Serology; Department of Entomology with sections of Field and Experimental Entomology of virus infections; Department of Zoology for investigation of vertebrate reservoirs of viruses: and a developing Department of Field Epidemiology, the VRC approaches a capacity capable of tackling an increasing number and variety of problems. A primary effort has been to integrate the various sections and departments into a combined attack on the various virus problems being investigated as the most expeditious and effective method of solving such problems. The account of results of these investigations which follows is not, therefore, entirely separated according to departmental or sectional efforts and interests, but as total progress on a given problem.

LABORATORY DEVELOPMENT.

In order to extend the spectrum of techniques for virus isolation and examination of agents from a larger variety of sources, technical staff and working facilities have been significantly expanded during the latter part of 1955. These developments include construction of an additional isolated preparation cubicle for the exclusive handling of materials from the field to avoid the potential risk of cross contamination in the preparation space where already established strains are handled. A cold room has been installed for the exacting temperature conditions required by hæmagglutination inhibition tests soon to be undertaken by the newly established section of serology which is already engaged in complement fixation testing of sera for antibodies to neurotropic viruses. A schedule of production of complement-fixation test antigens and control antisera for neurotropic viruses and other strains currently being studied at the VRC is already being implemented to provide a ready stock of such antigens for reference testing.

In the absence of any indigenous supply of solid carbon dioxide (dry ice) in India for proper storage of viruses at a low temperature of –70°C., a mechanically operated low temperature deep-freeze, maintaining a temperature of –47° C., has been secured and installed for preservation of a growing reference collection of viruses of interest and importance to India and the South Asian Region. Fully equipped tissue culture facilities for use of monkey kidney cells for virus isolation have been established and utilized particularly for the isolation of enteric viruses as described below. Development of chick embryo cell tissue culture has been inaugurated and soon should serve as a substantial expansion of routine tissue culture technique for isolation and study of viruses non-pathogenic to monkey kidney cells. This may be particularly useful in the study of arthropod-borne neurotropic viruses many of which are known to be pathogenic to cells of avian origin.

Steps have been taken toward development of library facilities of the VRC to include complete files of foreign journals and books dealing with microbiological problems as far back as 1940. Present journal files began in 1952 with establishment of the VRC.

MOUSE COLONY AND OTHER LABORATORY ANIMALS.

The mouse colony continues to be the backbone of investigations at the VRC. The development of tissue culture facilities broadens the spectrum of virus isolation possibilities and will, in time, take over some of the work for which mice are now used. However, confirmation and study of tissue culture isolations requires additional mice and with increase in numbers of virus isolations, added demand is made on mouse production.

The breeding output (Table I) was generally satisfactory in the cooler months of the year, but, as observed previously, the production decreased drastically in the period April to July as a result of summer temperatures. The critical temperature appeared to be a mean maximum daily temperature of 92 °F. or higher.

The necessary use of almost all litters born in the hot season for necessary passages and virus isolation depletes the stock of breeding females without allowing a sufficient number of infants to mature for replacement. Therefore, even after climatic conditions become more favourable for breeding in June, two or three months are required for production and maturation of additional females for replenishing and replacement and this only with continued restriction of mouse use for virological procedures. An attempt will be made to avoid this summer decrease in mouse breeding by installation of an air-conditioned mouse mating room in the near future.

The cotton rat colony, which was established in 1953 as the first in India, has done well and progeny have been delivered to other institutions for establishment of additional colonies for scientific work. Attempts to breed golden hamsters have, however, been unsuccessful.

Rhesus monkeys ordered from a private supplier have been of seriously inferior condition; eight of the last twenty delivered died of non-specific causes or tuberculosis shortly after delivery.

TABLE I.

Mouse colony report from 26th September 1954 to 3rd December, 1955.

Date	BREEDING				Females replaced (virgins)	DISCARDED		Litters pro-duced	Litters issued	Young mice pro-duced	Young mice issued
	No. of boxes	Males	Females	Total		Males	Fe-males				
26th Sept. to 30th Oct., 1954	420	420	2,779	3,199	556	0	0	959	721	2,628	1,030
31st Oct. to 27th Nov., 1954	420	420	2,494	2,914	704	0	0	998	672	3,538	1,792
28th Nov. to 25th Dec., 1954	420	420	2,093	2,513	658	0	0	1,148	744	670	2,344
26th Dec., 54 to 29th Jan., 1955	420	420	2,104	2,524	1,159	0	0	1,190	752	1,943	1,396
30th Jan. to 26th Feb., 1955	435	435	1,982	2,417	521	0	0	971	463	3,047	2,221
27th Feb. to 26th Mar., 1955	438	438	1,481	1,919	19	0	0	935	431	3,203	1,363
27th Mar. to 30th Apr., 1955	447	447	2,119	2,566	1,010	0	16	627	321	2,421	2,336
1st May to 28th May, 1955	448	448	2,067	2,515	298	37	0	672	414	759	3,403
29th May to 25th June, 1955	448	448	2,036	2,484				605	379	766	818
26th June to 30th July, 1955	420	420	1,728	2,148	816	39	145	1,559	907	3,096	998
31st July to 27th Aug., 1955	420	420	1,847	2,267	779	105	13	724	396	2,694	2,439
28th Aug. to 24th Sept., 1955	473	473	2,923	3,396	1,778	0	49	782	909	2,184	1,762
25th Sept. to 29th Oct., 1955	488	488	2,514	3,002	901	5	78	1,633	982	3,108	454
30th Oct. to 26th Nov., 1955	740	740	2,844	3,584	1,136	22	0	1,095	588	4,214	3,428
27th Nov. to 3rd Dec., 1955	740	740	2,594	3,334	35	0	0	181	220	579	620

POST-GRADUATE COURSE IN VIROLOGY.

The entire facilities and staff of the Virus Research Centre were diverted from the regular investigative programme from the latter part of February until early April in preparation for and presentation of a Post-Graduate Course in Virology, given under auspices of The Indian Council of Medical Research and actually scheduled from March 14 through April 2, in facilities provided by the B. J. Medical College, of Poona University as well as the VRC. Experts in various fields of virology from different institutions and laboratories in India were invited to participate in giving lectures, teaching and guiding laboratory demonstration of various virological techniques. A list of these teaching participants is appended to this report.

Student participants in the course were selected from various teaching, research and hospital institutions in India and numbered twenty-two in all according to the following categories:

Teachers in medical colleges	15
Workers in research institutes	3
Hospital staff doctors	3
Public Health service doctor	1
Total ...	22

It is difficult to evaluate at the present time such a short but comprehensive survey course of scientific methods for the study of virus problems involving human health. The net result in accomplishments in this field by the participants remains to be observed. However, it is apparent that the knowledge disseminated will serve to promote better understanding of virus disease problems as they occur in India, and to promote an understanding of the scientific approach to investigation of such problems as well as the limitations involved. In this respect the value of equal expenditure on long term training of a few workers destined to become specialized virologists cannot be compared.

The subject matter of the three weeks course deal twith arthropod-borne neurotropic viruses as represented by yellow fever and West Nile, respiratory viruses as represented by influenza, enteric viruses as represented by poliomyelitis and Coxsackie; also presented were psittacosis and lymphogranuloma viruses, hepatitis viruses, smallpox, rabies, atypical pneumonia and the rickettsioses.

JAMSHEDPUR FEVER.

A. *Jamshedpur material.*

Evidence against an arthropod-borne virus etiology.—It was reported last year that attempts to isolate a virus from human blood, brain and CSF from cases diagnosed as Jamshedpur Fever, in Tata Main Hospital, Jamshedpur, failed. Attempts to isolate an agent from the commonly found blood sucking arthropods of the city also were unsuccessful. It was also reported previously that an extensive gammexane spraying campaign in Jamshedpur during the epidemic did not produce evidence of alteration in the course of the epidemic.

Mouse-neutralization tests for rise in antibody titre between acute and convalescent sera, as well as challenge testing of mice inoculated for virus isolation, carried out at the Virus Research Centre, gave entirely negative results for dengue, West Nile, Japanese B, sandfly fever and Sindbis viruses as a cause of the increased incidence of febrile illness seen in Jamshedpur from May to September 1954. The list of arthropod-borne viruses to which there were negative findings was extended to twenty* by additional complement fixation tests on fifteen sera from Jamshedpur Fever cases accomplished at the New York Laboratories of the Rockefeller Foundation by Dr. Jordi Casals.

Investigation of possible enteric virus etiology.—As early as the middle of July, before any of the above evidence had been conclusive, the epidemiological picture of the occurrence of cases in Jamshedpur suggested that it might be an infection produced by an enteric virus. By the middle of August, when evidence against an arthropod-borne cause was accumulating, attention was turned toward examination of this possibility. Nineteen stool specimens were collected from cases diagnosed as Jamshedpur Fever and eighteen from associates and contacts of cases. Suspensions of some of these stools were prepared and inoculated intraperitoneally (IP) and intracerebrally (IC) into infant mice for virus isolation.

The isolation of a Coxsackie A4 strain (P1065) from the stool collected on the fifth day of illness from a 1½ year old female child and evidence against it being the cause of Jamshedpur Fever has been reported previously. It was isolated again (P1095) from a stool of the same patient collected on the seventeenth day after onset of the illness, and later from specimens collected from two siblings (P1871 and P1872) who remained asymptomatic.

Utilization of mice and facilities of the VRC to study this strain and other Coxsackie viruses isolated in India for possible etiological relationship to Jamshedpur Fever, deferred further examination of all stools collected in Jamshedpur until 1955, when suspensions of the remaining stools were inoculated into mice. An additional strain (P1081) of what appears to be Coxsackie A, unlike any others available for reference at the VRC, was isolated from the 45 years old father of two children who died, reportedly, of symptoms similar to those of Jamshedpur Fever.

Mouse-neutralization tests with this P1081 strain against 1:10 dilutions of sera of a Jamshedpur school survey collection showed only nine of forty-five not protective against 3·5 logs LD_{50} of the virus. This indicates widespread incidence of neutralizing antibodies against this agent in children of Jamshedpur who gave no history of febrile illness during the epidemic.

Acute sera of six children who died within a few hours of onset of the illness all neutralized the virus as did the nine close contacts of these two sibling children who died. Two pairs of acute and convalescent sera of two other children who became ill in the family, but survived, all neutralized the agent. A serum specimen taken from the father who yielded the virus in his stool a few hours after the stool was collected neutralized at least two logs LD_{50} of the virus.

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*Additional viruses tested: Eastern equine encephalitis, Venezuelan equine encephalitis, western equine encephalitis, Semliki Forest, St. Louis, Ilheus, Russian Far East, yellow fever, Zika, Colorado tick fever, Bunyamwera, Wyeomia, California and Anopheles A.

There is so far no evidence that the P1081 strain is in any way associated with the etiology of Jamshedpur Fever. In interpreting serological tests for enteric viruses it should always be kept in mind that high titre specific neutralizing antibodies have been frequently observed in sera of patients at the onset of symptoms of enteric virus infections.

Results of virus isolation attempts from other Jamshedpur stools inoculated into mice were negative. However, when well equipped tissue culture facilities had been established at the VRC in October and November 1955, the stool specimens were reexamined in monkey kidney cell cultures and yielded three additional strains (P1079, P1089 and P1098) as shown in Table II, which lists all strains so far isolated from the Jamshedpur stools.

Two of these strains are of particular interest because they are from contacts of the two children who died; one is from a sibling while actually suffering from a diagnosed case of Jamshedpur Fever. Those strains appear to be distinctly different from P1081, discussed above as isolated from the father of the children, because they produce cytopathogenic effect in monkey kidney cells. Because these strains have recently been isolated, testing of their etiological relationship has not been accomplished. It is of interest that the B Coxsackie viruses, which have been involved in CNS disease elsewhere and are discussed below, produce cytopathogenic effect in monkey kidney cells.

Serological epidemiology of Coxsackie virus infection in Jamshedpur.— Early in consideration of a possible enteric virus etiology of Jamshedpur Fever, efforts were made to test paired acute-convalescent sera and series of survey sera collected from different population groups of Jamshedpur against enteric viruses isolated in India. Mouse material containing eleven strains of Coxsackie viruses, isolated from human stools and flies, were obtained from Dr. D. D. Banker, then Assistant Professor of Pathology, S. G. S. Medical College, Bombay.

These strains had not been compared nor typed. Considerable preliminary work was involved in their study before they could be used as test agents. Ten were viable and they proved to be of five different types of Coxsackie group A. No rise in titre of antibodies to any of them could be demonstrated in the paired acute-convalescent serum specimens as shown in Table III.

The results of neutralization tests on the acute sera of this paired series, however, gave some interesting and perhaps significant information about the extent and prevalence of enteric virus transmission in the population of Jamshedpur. Results of these tests are given in Tables III and IV. It should be noted that the distribution of antibodies to the various viruses differs in varying degree, certain ones markedly so. This indicates differing endemic and epidemic behaviour of the agents. It is significant in that it demonstrates that fæcal borne virus transmission is common, widespread and occurs often at an early age in the population. This is clear evidence that if Jamshedpur Fever was caused by an enteric virus, the mechanism was present for its rapid dissemination, particularly in a non-immune population.

TABLE II.

Viruses isolated from human stool specimens collected at Jamshedpur.

Strain	Age and sex of donor	Category	Date of collection	Date of inoculation	Pathogenic infant mice	Pathogenic adult mice	Cytopathogenic MKTC	Seitz filtration	Ether resistance	Related or identical to	Re-isolation	Immune	Identity
P1065	1½F*	Acute	28–8–54	5–9–54	+	—	...	+	+	Coxsackie A_4	+	+	...
P1079	3 M	Acute	4–9–54	17–11–55	...	...	+	...	...	...	+	...	...
P1081	45 M	Contact	8–9–54	14–5–55	+	—	—	+	+	None of VRC Coxsackie A	+	+	Coxsackie A
P1089	12 M	Contact	8–9–54	17–11–55	...	...	+	...	...	...	+	...	...
P1095	1½F*	Convalescent	14–9–54	21–9–54	+	—	—	...	...	P1065	+	...	Coxsackie A_4
P1098	12 M	Acute	18–9–54	17–11–55	...	...	+	...	...	...	...	...	...
P1871	8 M	Contact	25–9–54	29–4–55	+	...	...	...	...	P1065	+	...	Coxsackie A_4
P1872	M	Contact	25–9–54	5–2–55	+	—	...	...	...	P1065	...	...	Coxsackie A_4

*From the same patient.

Blank spaces indicate that the result is under investigation or not yet done.

TABLE III.

Paired sera from 59 cases of Jamshedpur Fever—Results of neutralization tests with seven strains of Group A Coxsackie virus, omitting inconclusive results.

P No.	1065		1559		1562		1563		1564		1568	
Dalldorf Type	A 4		A 12		A 14		A ?		A 3		A-4—Prob.	
Age	*P/T	C/T†	P/T	C/T	P/T	C/T	P/T	C/T	P/T	C/T	P/T	C/T
0–4	6/8		4/8		4/8		6/7	0/1	2/8	0/4	6/8	
5–9	2/3		7/10	0/1	7/10	0/1	6/8		5/8		9/9	
10–14	11/12		6/12	0/2	9/12		9/12		6/12	0/4	11/12	
15–19	8/9		6/9	0/2	8/10		8/10	0/2	5/10	0/3	7/9	
20–29	10/11		8/11	0/1	5/11	0/1	8/11	0/1	4/10	0/5	10/11	
30–39	6/6		2/8	0/2	2/5		7/7		5/8	0/3	5/7	
Total	43/49	0/0	33/58	0/8	35/56	0/2	44/55	0/4	27/56	0/19	48/56	0/0

*Number positive/number tested.

†Number of conversions from negative/number of pairs tested in which the acute phase serum was negative.

TABLE IV.

*Incidence of neutralizing antibody in very young children to the P-*1065 *strain of Coxsackie virus* (A-4) *in acute phase sera of cases of Jamshedpur Fever.*

Age in years	Number of sera Post./Test	Per cent positive
0 to 0·5	3/3	100
0.5 to 1	3/9	33
1	12/29	41
2	5/11	45
3	5/9	55
4	6/7	85
5	7/9	78

The possible rôle of Coxsackie B viruses.—Enteric viruses resistant to inactivation by ether and productive of myositis and encephalitis primarily in infant mice only have been identified as the cause of epidemic pleurodynia (Bornholm's Disease), aseptic meningitis and other central nervous system disease—including encephalitis in infants—and have been termed Group B Coxsackie viruses by Dalldorf. They have occasionally been isolated from CSF. The experience with cases of CNS disease due to agents, particularly type B3, of this group in widely separated places over the face of the earth (i.e., Australia, South Africa, Canada, United States, Argentina and Sweden) was considered early in the Jamshedpur Fever investigations. Since no known isolation of a B type Coxsackie virus had been made in India, attention to these agents was deferred until other Indian viruses had been tested.

On a visit to Dr. Gilbert Dalldorf's laboratory of the New York State Public Health Department in Albany, arrangements were made to secure type strains of the B group and typing sera for nineteen A group Coxsackie viruses. These arrived in India by air early in September. All four strains were passed and found to be viable but of low titre. Since Coxsackie CNS disease has most often been attributed to the B3 type, this was selected first for study. Several passages in mice were required before sufficient titre was attained for neutralization testing of Jamshedpur sera.

Four of six acute sera drawn from cases which died within 36 hours of onset of illness, tested in 1:10 dilution against Coxsackie B3, showed neutralizing antibodies. Seven of thirteen sera collected from contacts of fatal cases and tested in the same dilution contained similar antibody to the virus. The incidence of B3 antibodies in the sera of clinically unaffected population groups of Jamshedpur and rise in titre in paired acute-convalescent sera of cases have not yet been tested.

It is worth noting, however, that in Sydney, Australia, where pooled human serum specimens collected in each year since 1941 were available for testing, no antibodies to the B3 Coxsackie type of encephalitis virus were present until 1951, just prior to January 1952, when there was an explosive outbreak of encephalitis due to viruses immunologically related to B3. Following this outbreak the incidence of specific antibodies increased to high levels. With reference strains of Group B Coxsackie viruses now available at the VRC, serological examination for specific B group antibodies will be intensified in sera collected in Jamshedpur.

Orphan virus tests.

No avenue for seeking information on a possible enteric virus etiology of Jamshedpur Fever has been ignored. Serum specimens were sent to Dr. J. L. Melnick at the Virus Laboratories of Yale University School of Medicine in New Haven, Connecticut, and to Dr. Albert Sabin at Children's Hospital Research Foundation in Cincinnati, Ohio, for testing against the growing type collections of ' orphan ' viruses. These were given the name ' orphan ' because they were originally isolated from human stool specimens in tissue culture cells, primarily of the monkey kidney cell type, and have not been found pathogenic to the usual laboratory animals such as mice and rhesus monkeys.

Results of these tests of acute and paired acute-convalescent Jamshedpur Fever sera against orphan viruses used show no conclusive evidence so far that any of them are associated with the etiology of the disease. However, demonstration of antibodies to certain strains in some sera indicates the cosmopolitan nature of these viruses, most of which were originally isolated in the United States, but appear to be active elsewhere in the world.

An infectious or toxic non-virus etiology of Jamshedpur Fever has been considered, but since this extends to non-virological fields it is not considered here.

(B) *Delhi Material.*

Investigation of the etiology of Jamshedpur fever-like (" mystery disease ") cases in Delhi:

Cases of encephalitis in children, which clinically appear similar if not identical to Jamshedpur Fever, have been observed and studied in the Pediatrics Ward of Irwin Hospital, New Delhi, by Dr. P. N. Taneja, Pediatrician and Dr. A. S. Sidhu, Indian Council of Medical Research, Research Fellow assigned for special investigation of the problem. These are cases of " Mystery Disease ", so well publicized in newspapers in 1954, after reports of the Jamshedpur Fever outbreak. The epidemic in the Delhi area in 1954 appears to have reached its peak later in the season than in Jamshedpur and sporadic cases have occurred in children throughout the year with a short upswing in incidence last August with eight cases and four deaths in eight days.

Stool specimens and convalescent sera have been collected from these cases almost continuously since June, and forwarded on wet ice in a thermos to the VRC for virological and serological investigation. Results of attempts to isolate virus from stools are given in Table V. It is noted that ten stool isolates have been recovered, eight from stools collected since early in June. Three or four of the ten appear to be types of " orphan " viruses described previously.

Comparison of these strains has been commenced. Many show low titre in mice so far. Others await availability of mice for study, most of the mouse production since October having been diverted for investigation of another encephalitis problem described below. A very few early acute sera have been obtained from recent cases and they will be tested along with convalescent sera for rise in antibody titre against homologous and heterologous viruses when the strains are in high enough titre.

In considering the infant mouse pathogenic monkey kidney cell cytopathogenic strains, B3 Coxsackie possibilities must be excluded. This is being done by neutralization testing with a specific B3 Coxsackie antiserum, which has been prepared in mice, as and when an adequately titred stock is achieved for each strain. However, it should be noted that in only six of twenty-three sera (mostly convalescent) of cases observed at Irwin Hospital, gave evidence of antibodies to B3 Coxsackie virus when tested in 1:10 dilution.

Serological epidemiological studies await collection of survey samples from the population of New Delhi, similar to collections made in Jamshedpur.

ARTHROPOD-BORNE VIRUS ANTIBODIES IN HUMAN SERA OF EASTERN INDIA:

Jamshedpur, Bihar.—Testing of sera of Jamshedpur Fever patients for an arthropod-borne virus etiology of the disease produced negative results as described previously. However, there were antibodies to such agents in some of the sera which implied that some arthropod borne viruses were active or had been in the city in the past. This encouraged further investigation not only of sera of acute cases, but various survey collections, in mouse neutralization tests against dengue I, West Nile, Japanese B and Sindbis viruses. No serological investigation of exposure of people

TABLE V.

Viruses isolated from human stool specimens collected at Irwin Hospital, New Delhi.

Strain	Age and sex	Category	Date of collection	Date of inoculation	Pathogenic infant mice	Pathogenic adult mice	Cytopathogenic MKTC	Seitz filtration	Ether resistance	Related to or identical to	Re-isolation	Identity
P1116	F	Acute	15–9–54	1955	—		+‡				—	Orphan
P1118	F	Acute	16–9–54	1955	—		+‡				+	Orphan
P2344	11/12 M	Acute	16–6–55	22–6–55	+*		+‡			None of VRC Coxsackie A	—	
P2747	9/12 M	Acute	27–8–55	19–9–55	—		+‡				+	
P2751	2½ F	Acute	5–9–55	24–9–55	+*		+‡				+	
P2752	1½ M	Acute	5–9–55	16–9–55	+†		+†				+	
P2929	8/12 F	Acute	8–9–55	3–10–55	+‡		+				+	
P2930	10/12 F	Acute	15–9–55	28–9–55	+‡	—	—	+	+	None of VRC Coxsackie A		
P2976		Acute	13–10–55	21–10–55	+‡		—					
P3046		Acute	19–10–55	7–11–55			+‡				+	

* Pathogenic to infant mice after two passages in tissue culture. Original stool negative in mice.

† FN2-One strain each in tissue culture and mice from original stool. Identity of the two not tested. 2nd passage tissue culture fluid not pathogenic to mice.

‡ Original isolation.

in this area of India had ever been accomplished previously. Results with dengue I and West Nile are tabulated in Tables VI and VII:—

TABLE VI.

Results of neutralization tests with human sera from Jamshedpur and dengue I and West Nile virus.

Category	Age in years	DENGUE I				WEST NILE			
		Neg.	Inc.	Pos.	Tot.	Neg.	Inc.	Pos.	Tot.
Acute-phase sera from cases of Jamshedpur Fever	1–4	5	2	1	8	6	2	0	8
	5–9	2	1	0	3	7	2	1	10
	10–14	1	1	10	12	2	3	7	12
	15–19	2	2	6	10	3	3	4	10
	20–29	1*	0	10	11	3	4	4	11
	30–39	0	0	8	8	0	2	6	8
	Totals:	11	6	35	52	21	16	22	59
	Per cent:	21	12	67		36	27	37	
Survey	5–14	1	0	2	3	2	0	1	3
	15–19	3	1	5	9	6	2	1	9
	20–29	2	2	14	18	6	7	5	18
	30–39	1	1	5	7	1	3	3	7
	40–49	1	0	8	9	2	2	5	9
	50	0	0	10	10	4	2	4	10
	Totals:	8	4	44	56	21	16	19	56
	Per cent:	14	7	79		37	29	34	

*Nurse from Travancore-Cochin.

TABLE VII.

West Nile virus neutralization tests on Jamshedpur sera of various categories.

	Negative	Inconclusive	Positive	Total
0–4	14	4	1	19
5–9	27	5	0	32
10–14	8	6	8	22
15–19	12	10	9	31
20–24	10	8	9	27
25–29	5	13	7	25
30–39	3	8	13	24
40–49	3	3	16	22
50	4	4	4	12
Totals ...	86	61	67	214
Per cent ...	40	29	31	

It is apparent from these analyses that the incidence of dengue I antibodies is high and there is a lower but significant incidence of immunity to West Nile virus. A remarkably large number of inconclusive results, usually in the range of 30 per cent were observed in each of the early tests where the LD_{50} ranged from 1·7 to 2·6 logs. This was at first attributed to the age of the test mice (six to eight weeks old). But when three and four weeks old test animals were used, a similar incidence was noticed. In analysing a large number of results consolidated from a number of tests, as shown in Table VII, a similar distribution was observed; an increased incidence of positives being noted probably due to an increased proportion of adult human sera in the total analyses.

This seemed to indicate a number of sera with either a low titre of specific antibodies to West Nile virus or antibodies to a related antigen or both. Previous experience in an endemic area of West Nile virus infection in the Nile delta of Egypt led to the expectation of a higher titre neutralization capacity with a low percentage of inconclusives if the antibodies were specific for West Nile virus.

In considering the possibility of a related virus as a cause of this phenomenon it seemed reasonable to investigate the prevalence of Japanese B encephalitis virus antibodies because of its close immunological relationship to West Nile. No such tests for this virus were known to have been carried out on human sera collected in eastern India. The knowledge of Japanese B endemicity in Malaya, Thailand and Burma, suggested the possibility that its range might extend into eastern India where Jamshedpur is situated. The serum collecting programme in Calcutta and environs to obtain information on this problem is described below. However, it should be noted here that later neutralization tests for Japanese B in Jamshedpur sera indicate presence of a significant incidence of antibodies to this virus although it appears to be markedly less than for West Nile. In an industrial area, such as Jamshedpur, there is a large floating population of labourers and the possibility of exposure of some of these persons to virus infection elsewhere should be considered. Dengue and West Nile viruses cause non-fatal febrile illnesses of high morbidity where they have been studied clinically. Japanese B also produces febrile illness, not uncommonly resulting in fatal or maiming encephalitis. Therefore, further definition of specificity of these serological reactions seems indicated and, when possible, actual attempts to isolate the virsus producing these antibody responses in the localities where they occur.

Related occurrence of neutralizing antibodies to dengue I, West Nile and Japanese B viruses in human sera of Jamshedpur.—On examination of results given in Table VIII, it is remarkably apparent that all sera with sufficient quantity of antibody to neutralize two or more logs of West Nile virus also neutralized dengue I. Those sera with a lesser amount of West Nile neutralizing antibody, as indicated by inconclusive results, almost invariably had some neutralizing action against dengue I. But the converse was not demonstrated because there were a significant number of dengue I positive and inconclusive sera which were West Nile negative. It should be observed that all Japanese B positive sera were either positive or inconclusive to West Nile, but in lesser numbers which is the reverse of what has been found in Japanese B endemic areas in S.-E. Asia.

TABLE VIII.

Comparison of incidence of West Nile neutralizing antibodies to dengue I neutralizing antibodies in sera collected in Jamshedpur.

(1) Acute phase sera collected from patients diagnosed as Jamshedpur Fever. Therefore, biased for younger age groups.

Sera inactivated at 56° C. for one hour prior to addition of Dengue I virus.

	DI +	DI inc.	DI —	Total	Per cent
WN + ...	17	1	0	18	41
WN Inc. ...	10	2	2	14	31
WN — ...	3	7	2	12	28
Total ...	30	10	4	44	...
Per cent ...	68	23	9	...	...

(2) Survey sera collected from adults.

Sera not inactivated.

All sera were also tested for Japanese B antibodies.

Numbers in parentheses are Japanese B positive.

	DI +	DI inc.	DI —	Total	Per cent
WN + ...	22 (6)	0	0	22 (6)	39
WN Inc. ...	9 (1)	3 (1)	1	13 (2)	23
WN — ...	14	1	6	21	38
Total ...	45 (7)	4 (1)	7	56	...
Per cent ...	80	7	13	...	...

In the original antibody survey of localities in western India (Smithburn, Kerr and Gatne, 1954) it was noted that several places which had high incidence of dengue I antibodies also had a significant number of West Nile positive sera. There were some localities of very small numbers tested where there was an occasional West Nile positive, but none to dengue I. In localities with larger numbers tested for both, some show higher incidence for West Nile antibodies.

Data on this problem obtained in Trinidad, an area where existence of West Nile infection has not been demonstrated, show that of 91 sera tested against both dengue I and West Nile, 40 were positive, 20 inconclusive, and 31 negative to dengue I. Of the 91 sera, seven were West Nile positive, six of them also being dengue I positive and one negative. Of six West Nile inconclusives, half were dengue I positive and half inconclusive.

Further investigation of this phenomenon will be continued on sera collected elsewhere in India. The repeated appearance of large numbers, up to 1/3, of inconclusives in West Nile neutralization tests, and conversion of these to negative by addition of a log or less of additional virus, indicates low titre of antibody which does not appear to be due to Japanese B virus infection and appears unusual for specific West Nile infection. That so many of the West Nile positive and inconclusive sera of Jamshedpur should consistently have antibodies to dengue 1 is considered to be evidence against double infection. This becomes of considerable potential importance if it is considered evidence of some other mechanism of virus transmission which may play a rôle in blocking dissemination of more serious arthropod borne viruses such as yellow fever.

Calcutta and environs.—The phenomenon of large numbers of Jamshedpur sera showing small amounts of neutralizing antibody against West Nile virus has been discussed above and inquiry as to its possible relationship to Japanese B virus infection. For practical reasons, collection of avian and human sera in urban and rural areas of Calcutta in West Bengal were planned and accomplished before the Jamshedpur sera could be tested specifically against Japanese B encephalitis virus.

Three collection points were selected: (1) Diamond Harbour at the junction of the Hooghly river with the tidal flats of brackish water from the Bay of Bengal, (2) The urban area of Calcutta, and (3) Maricha, a farming community on the Gangetic plain about 30 miles north of Calcutta, far away from any ecological association with the sea. These collections, which were accomplished in January, were arranged with the help of Dr. B. C. Das Gupta, Director of Public Health, West Bengal, and Dr. R. N. Chaudhuri, Director, Calcutta School of Tropical Medicine. In addition, Dr. Chaudhuri gave full facilities of the School for separation of sera and seconded Dr. K. N. Neogy, Assistant Professor of Bacteriology, to help with some of the blood collections.

When these sera arrived at the VRC, Poona, they were tested for neutralizing antibodies to West Nile, Japanese B, and dengue I viruses. Results of these tests are recorded in Table IX. A number of these tests were set up months after collection of the sera. Neutralizing antibodies to certain viruses, including West Nile and Japanese B, are known to diminish in sera after several months storage apparently due to loss of a labile factor. The neutralizing effect of such sera, however, can be partially or completely restored by addition of a fresh serum factor in non-immune human or monkey serum drawn just before testing. Consequently, those sera tested against these two viruses more than three months after collection had the fresh serum factor added (·15 c.c. test serum + ·3 c.c. fresh monkey serum + ·15 virus). Control virus titrations were done with ·45 c.c. of 75 per cent BAPS + ·15 virus.

It is obvious from analysis of the tests that there is minimum evidence of activity of Japanese B or related viruses in the Calcutta area of eastern India. It is, therefore, unlikely that the Japanese B endemic area of South-east Asia extends as far as West Bengal. How far west it does continuously extend and whether it reaches any part of north-eastern India remains to be determined.

It is interesting to observe the evidence of high endemicity of dengue I infection in Diamond Harbour where there is solid immunity in

TABLE IX.

Antibodies to certain arthropod-borne viruses in Calcutta and environs.

	DENGUE I					WEST NILE					JAPANESE B				
	—	Inc.	+	Total	Per cent +	—	Inc.	+	Total	Per cent +	—	Inc.	+	Total	Per cent +
Diamond Harbour															
0–4	0	0	6	6	100	8	0	0	8	0	8	0	0	8	0
5–9	2	0	2	4	50	5	0	0	5	0	5	0	0	5	0
10–14	7	0	5	12	41	14	0	0	14	0	13	0	1	14	7
15–19	2	0	7	9	78	10	0	0	10	0	10	0	0	10	0
20–29	0	0	5	5	100	8	2	0	10	0	8	2	0	10	0
30–39	1	0	2	3	67	5	0	1	6	17	6	0	0	6	0
40–49	1	0	4	5	80	7	0	1	8	13	7	0	1	8	12
50+	0	0	1	1	100	1	1	1	3	33	1	0	2	3	66
Total ...	13	0	32	45	73%	58	3	3	64	5%	58	2	4	64	6%
Calcutta (filariasis)															
0–4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
5–9	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
10–14	...	...	...	...	...	2	0	0	2	...	2	0	0	2	...
15–19	2	0	2	4	...	5	2	1	8	...	7	0	1	8	...
20–29	1	0	2	3	...	3	0	1	4	...	3	0	1	4	...
30–39	...	...	...	...	...	0	0	1	1	...	0	0	1	1	...
40–49	0	0	1	1	...	0	0	2	2	...	2	0	0	2	...
50+	0	0	1	1	...	0	0	1	1	...	0	0	1	1	...
Total	3	0	6	9	67%	10	2	6	18	33%	14	0	4	18	22%

TABLE IX.—(*Concld.*)

Antibodies to certain arthropod-borne viruses in Calcutta and environs.

	DENGUE I					WEST NILE					JAPANESE B				
	—	Inc.	+	Total	%+	—	Inc.	+	Total	%+	—	Inc.	+	Total	%+
Calcutta															
0–4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
5–9	3	0	3	6	50	8	0	1	9	11	7	0	1	8	13
10–14	2	0	5	7	71	7	3	1	11	9	8	1	1	10	10
15–19	1	0	5	6	83	5	1	3	9	33	3	1	1	5	20
20–29	3	0	5	8	63	9	3	3	15	20	10	0	2	12	
30–39						1	0	1	2	50	1	0	0	1	0
40–49						1	0	0	1	0	1	0	0	1	0
50+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total	9	0	18	27	67%	31	7	9	47	19%	30	2	5	37	14%
Maricha															
0–4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
5–9	5	0	2	7	29	9	1	0	10	0	10	0	0	10	0
10–14	5	0	0	5	0	5	0	0	5	0	5	0	0	5	0
15–19	4	0	1	5	20	6	0	0	6	0	6	0	0	6	0
20–29	5	0	1	6	17	7	1	0	8	0	8	0	0	8	0
30–39	1	0	1	2	50	7	0	0	7	0	6	0	0	6	0
40–49	3	0	2	5	40	6	1	0	7	0	5	1	1	7	14
50+	2	0	0	2	0	1	2	2	5	40	2	0	2	5	60
Total	25	0	7	32	22%	41	5	2	48	4%	42	1	4	47	8%

Discrepancies in total numbers of sera tested against each virus from one locality result from exclusion of unsatisfactory tests. These sera must be retested and added to the totals before the results can be considered comparable. In the Calcutta collection, eighteen sera were drawn from persons infected with bancroftian filariasis to determine if evidence of greater exposure to arthropod borne viruses could be demonstrated.

the very young children as well as a high incidence of antibodies in the other age groups. The incidence of West Nile and Japanese B antibodies is so low that it offers no present basis for examination of the West Nile—dengue I relationship discussed above.

The marked difference of dengue I immunity in the farming community of Maricha, fifty miles inland from Diamond Harbour is of significance. The minimal incidence of West Nile and Japanese B antibodies appears to be of little significance, mostly occurring in the older age groups in people who could possibly have been exposed elsewhere, or at some time in the distant past.

The urban area of Calcutta shows a fairly high incidence of dengue I antibodies which would seem to indicate that infection with this or a related virus is not uncommon. The small, but significant incidence of antibodies to West Nile and Japanese B viruses may indicate occasional activity of one of these or a related agent, but the real significance of this observation remains to be explored.

Of 35 crow sera collected in the three localities two showed presence of neutralizing antibodies to West Nile virus. Significance of this observation is at present unknown.

SINDBIS VIRUS ISOLATIONS.

The last annual report listed eight unidentified strains isolated at the VRC. Five of these, i.e., B322/23/24 wagtail spleens, Poona; B571 and B572, hill mynah spleens, Cochin; A1033(*b*), pool of *Culex spp.* mosquitoes, North Kanara; and A1036, pool of Bdellonyssus mites, North Kanara; have now been identified as strains of Sindbis.

The first isolation of Sindbis virus was made in 1952 near Cairo, Egypt. Between 1952 and 1955, 13 strains were isolated in Egypt; 11 from *Culex* mosquitoes, one from *Anopheles* mosquitoes, and one from the blood of a juvenile crow.

Detailed characteristics of the Indian strains have been described in a paper awaiting publication and will, therefore, only be briefly mentioned here.

Host range.—All strains are pathogenic only to infant mice and have an incubation period of three to six days. The virus propagates in three weeks old mice without manifesting symptoms and can be recovered from the brain four days after IC inoculation of the virus. The susceptibility of the mice decreases rapidly with increase in age.

Yolk sac inoculation of embryonated eggs produces death within one to three days. Subcutaneous (SC) inoculation of chicks and IC and SC inoculation of *rhesus* monkeys results in detectable amounts of virus circulating in the blood and formation of specific neutralizing antibodies.

Physical characteristics.—Filtration through Seitz EK filter pads and lyophilization did not markedly decrease the titre.

Identification.—Two of the strains, A1036 and B572, were identified by complement-fixation and hæmagglutination-inhibition tests by Dr. Jordi Casals at the Rockefeller Foundation Virus Laboratories in New York. The remaining three were identified by cross neutralization tests accomplished at the VRC.

Serology.—Mouse-neutralization tests were done mainly with strain A1036. The labile factor mentioned previously, which is also important in tests for antibodies to Sindbis virus, was provided by addition of non-immune *rhesus* monkey serum to the sera to be tested.

Of 174 human sera tested, five (2·7 per cent) were positive. These consisted of 4 of 43 (nine per cent) of Khadakwasla, a rural village near Poona, and one of 76 (1·7 per cent) from Devimane, North Kanara District, Bombay State. None of the 76 sera from Poona were positive.

All 57 *rhesus* monkey sera from the animal house of the VRC were negative and only one of eight horse sera positive. One of 20 (five per cent) crow sera collected around Poona was positive, while 19 fowl sera from the same area were all negative.

The isolation of these strains is the first significant evidence of the presence in India of any of serological Group A viruses as classified by Casals and Brown. This group includes the three equine encephalitis viruses, Semliki Forest and Sindbis viruses. These isolations in India, following those in Egypt, indicate that Sindbis virus has a natural distribution over extensive geographical areas. The isolation from chicken mites is the first to be reported from mites.

The effect or importance of Sindbis infection in man is not yet known. Possibly Sindbis, like many arthropod borne viruses, is primarily an avian or arthropod virus only occasionally infecting man. This concept seems to be supported by these isolations; three strains from bird spleens, one from chicken mites and one from mosquitoes known to feed on birds and mammals other than man.

MOUSE PATHOGENIC AGENTS ISOLATED FROM GOAT TICKS COLLECTED IN ORISSA AND (POONA) BOMBAY.

In June 1954, Dr. G. Biswal, then Veterinary Research Officer for Orissa and presently Principal of the Veterinary College in Cuttack, spent a week at the VRC endeavouring to learn a virological approach to investigating the etiology of lumbar paralysis in goats and sheep, a veterinary problem which arose when the Veterinary Department of the Orissa Government imported large black beetal goats from the Punjab to increase the size and quality of small Orissan stock. In October, 1954, Dr. Biswal collected *Hæmaphysalis parva* ticks from animals of a hybrid herd at Bhanjanagar in which lumbar paralysis was observed. Some of these ticks were dispatched to the VRC for examination. In an effort to aid him at the beginning of his investigation of this problem, from the virological point of view, pools of these ticks were inoculated IC into mice. Simultaneous testing of goat tick pools, collected in Poona, was carried on.

Four non-bacterial infectious agents, pathogenic to mice, were isolated from a total of sixty pools comprising of 645 ticks; two (G619 and G690) from *Hæmaphysalis parva* collected in Bhanjanagar, Orissa, and two (G673 and G700) from *Hyalomma ægyptium* collected in Poona, Bombay. Details of these isolations are listed in Table X.

Characterization of the strains.—The G619 strain was pathogenic to both infant and adult mice, attaining a titre of greater than $10^{7\cdot5}$ in infants and $10^{6\cdot1}$ in adults by the third passage. G690 showed the marked difference

TABLE X.

Strains from ticks of Orissa and Poona.

Strain	Species	Host	Date of collection	Date of inoculation	Pathogenic infant mice	Pathogenic Adult mice	Seitz filtration	Ether Resistance	Lyophilization	Related to or identical with	Reisolation	Immune serum
G 619	Hæmaphysalis sp. probably parva (19)	Healthy goats Bhanjanagar Orissa	5–11–54	12–11–54	+	+	+4·7 log titre loss		+	None in this table	—	+
G 673	Hyalomma ægyptium (10)	Healthy goats Kondwa, Poona District	14–12–54	15–12–54	+	—	+1·5 log titre loss	+	+	None in this table	—	+
G 690	Hæmaphysalis parva (7)	Paralyzed goat, Bhanjanagar, Orissa	18–12–54	25–12–54	+	+	+ 4·2 log titre loss	+	+	None in this table	—	+
G 700	Hyalomma ægyptium (4)	Healthy sheep, Wanowri, Poona.	27–12–54	29–12–54	+	—	+ 3·8 log titre loss		+	None in this table	+	+

of more than four logs LD$_{50}$ in titre between infant and three weeks old mice. G673 and G690 were pathogenic only to infant mice.

The incubation periods of G619, G690 and G673 were three to five days; that of G700, six to eight days. All the agents passed through a Seitz EK filter at pH 7·1-7·2. Three of them, however, lost about four logs LD$_{50}$ in the filtration. When exposed to 20 per cent anhydrous ethyl ether after an initial centrifugation at 1500 r.p.m. for fifteen minutes, G690 and G673 titred $10^{6\cdot1}$ and $10^{5\cdot0}$, respectively, as compared to $10^{7\cdot4}$ and $10^{6\cdot4}$ of the controls. All strains were shell frozen and lyophilized without marked loss of titre although the potency of the dried preparations deteriorated rapidly. Hyperimmune mouse sera were successfully prepared by IP inoculations of the agents once weekly for three weeks.

Studies for identification.—Table XI gives results of cross neutralization tests of these strains with each other and with the A1036 strain of Sindbis. Though incomplete, the results indicate that all four strains are unrelated to each other.

TABLE XI.

Cross neutralization tests of the tick strains against one another and Sindbis strains (A1036).

ANTISERUM		STRAINS				
Strain	Animal	G619	G673	G690	G700	A1036
G619	Mouse M5828	3·5		0·3	0·2	*<0·5
G673	Mouse M5788	0·5	1·0 *1·7	0·4	—0·2	*>0·5
G690	Mouse M5651	0·3 0·4		2·1	0·2	*<0·5
G700	Mouse M5827	0·5		0·3	2·7	
A1036	Rhesus R51		*·7			*3·1 *>3·6

*With addition of fresh non-immune serum.

Col. S. L. Kalra of the Armed Forces Medical College tested the mouse immune sera against the ricketsial antigens of Q fever, Rocky Mountain spotted fever and murine typhus with negative results. Studies toward further description and identification of these agents continue.

Serology.—Most of the serological work was done with the G690 strain because it was derived from ticks collected from a paralyzed goat at Bhanjanagar. Sera of Bhanjanagar goats markedly lengthened the incubation period, resulting in prolongation of the average survival time (AST) as compared to sera from other localities. However, most of the test mice ultimately succumbed. This, phenomenon was observed with sera collected from both the imported and indigenous breeds of goats. The indigenous goats are not known to suffer from lumbar paralysis.

These serological results are given in Table XII which indicate that there is some sort of antibody response in almost all of the Bhanjanagar goats but not very evident in goats of the other two areas of Orissa, i.e., Keonjhar and Patha.

TABLE XII.

Results of neutralization tests of goat sera against G690.

Locality	Category	Number of sera tested	Mean AST of all	+	I	—
Poona	Indigenous goats	21	5·2	3	2	16
Wanowrie	Beetal goats	14	4·3	0	0	14
Keonjhar Orissa	Local indigenous goats	8	5·1	0	1	7
Patha, Orissa	Beetal goats	16	4·6	0	0	16
Bhanjanagar Orissa	Beetal goats	23	8·7	21	2	0
	Local goats	4	8·9	3	0	1

From the goat from which ticks were collected which yielded the G690 strain paired serum samples were obtained on the fourth day and three months after paralysis was observed. No increase in neutralizing capacity could be demonstrated because both sera apparently neutralized to an equal degree.

Paired sera from another affected goat, the first taken before onset of the paralytic illness and the second more than two weeks after paralysis gave similar results in that both apparently gave the same order of neutralization. Therefore, there is no evidence that G690 has any etiological relationship with lumbar paralysis in goats in Orissa and the symptomatology, if any, caused by this agent is unknown.

Specimens of two goats brains collected late in the illness and sent to the VRC from Cuttack, in unrefrigerated glycerine were negative for virus on inoculation into mice.

Human sera from Orissa and Bombay States have not yet been tested for antibodies to these agents. While this problem does not appear to be of importance to human health, and therefore, of primary interest to the VRC, it is another example of existence of infectious agents in arthropods about which nothing is known but deserving of more extensive investigation, not only from a veterinary, but a basic microbiological point of view.

KOLABA-POONA-THANA DISTRICTS (BOMBAY STATE) FIELD PROJECT.

Various field projects which could be undertaken at different localities have been under intermittent consideration since establishment of the VRC. Except for the three month field study in the Devimane area of

North Kanara District of Bombay State, organized at the end of 1953 by the former Deputy Director, Dr. Harald N. Johnson, which produced a number of virus strains and a new species of *Ixodes* tick, no long term intensive field investigation for defining virus problems of a populated area of definite dimensions and characteristics had been undertaken by the Virus Research Centre prior to July 1955. For various reasons, consideration was given to selection of an area reasonably convenient to the laboratory facilities, yet with varied enough ecological character to give promise of differential epidemiology.

On the basis of preliminary reconaissance in November 1954 and July 1955, an area about eighty miles square between Poona and the Arabian Sea was selected. This area not only contained a section of the Deccan Plateau, with which there was already some field, serological and entomological acquaintance, but altitudinal variation from over four thousand feet from sea-level, with evergreen, deciduous gallery, bamboo and scrub forests, mountains, foothills, riverine plain, brackish water estuary, seacoast, salt flats, and possibly limited perennially irrigated area, in addition to the one crop monsoon paddy farming terrain at various elevations with different fringe habitats throughout the area.

The southern half of the area is accessible by road branching southerly from the Bombay-Poona highway at Khopoli. Near the town of Pen, a rough road turns south over paddy farmed plain twenty-five miles to Indapur, crossing two rivers enroute. Twenty-three miles south of Indapur is Mahad, a large town of ten thousand people at the junction of the wide Savitri River with the tidal brackish water estuary draining 14 miles westward into the sea.

All of this area receives between 100 and 200 inches of rain which falls within four months of each year. Mahad is the rural-urban locality in the field area most distant from Poona (125 miles by either route in monsoon season) and with a large government clinic and daily State Transport bus service to Poona for courier handling of specimens, appeared to be the best place to establish our first field laboratory.

South and east of Mahad the road crosses the Savitri and twelve miles away turns east at Poladpur to approach the Western Ghats, where in 18 miles it winds and twists spectacularly up the precipitous cliffs, through wet forests and clinging mists of monsoon clouds in the rainy season, and through bright clear sky the remainder of the year, to Mahabaleswar, over 4,000 feet above sea level. Continuation eastward is a more gentle decline onto the Deccan Plateau and to the Satara-Poona Highway to complete the circuit.

Access by car to the seacoast from the Pen-Mahad road can be accomplished only during the dry season over cart tracks. Alibag and Murud, coastal towns parallel to towns and villages visited in the interior were reached by road from Pen via Alibag. The environment at Alibag and for some distance north as well as south to Revdanda, is coco-nut and areca palm lined sandy and rocky coastline. This narrow strip of dense vegetation is damp and shady, in distinct contrast to the dessiccated ladscape of the interior between December and May.

Serological survey collections.—As a preliminary step in evaluating the region for long term investigation, survey collections of human and avian sera were inaugurated early in August and continued with periodic visits to different localities. These sera were collected in Poladpur, Varandha, Mahad, Goregaon, Mangaon, Indapur and Wavediwali, Kolad and Bhira. Besides his general help all over the district in support of our investigations, Dr. M. A. Joglekar, Kolaba District Civil Surgeon was particularly helpful to us in obtaining serum collections from Alibag and Murud as Dr. B. K. Mahajan, District Health Officer, was in similar work in Pen and Karjat.

As these serum collections were made they were put up in neutralization tests against West Nile and dengue I viruses as a first step in obtaining information about possible endemic and epidemic virus problems in different localities. Results of these tests to-date are given in Tables XIII and XIV. With complement-fixation tests now being accomplished at the VRC and hæmagglutination inhibition testing to be developed in the near future, information on a wider spectrum of viruses should become available more rapidly.

The occurrence of significant incidence of West Nile neutralizing antibodies in Mahad, Goregaon and Mangaon—all situated on the Savitri River drainage—was as surprising as the complete lack of evidence of dengue I activity in the same area. On the basis of the preliminary serological survey in India (Smithburn, Kerr and Gatne 1954) it had been suggested that arthropod-borne virus activity was associated with areas of low rainfall, which this area certainly is not. Activity of West Nile virus or a related agent does not appear to have been recent, however, because none of the positive are under ten years of age. This raises the question of whether it periodically becomes epidemic in this area, and if, so from where does it come.

Another interesting observation is that the other localities that show little serological evidence of exposure to this type of virus are located but short distances away, in apparently similar environment surrounded by flat fields of paddy, except that large streams are not commonly found in association with such villages. The significance of this difference remains to be investigated either from the point of view of effect on arthropod vector populations or routes for introduction of infections by man or other migrating vertebrates such as birds.

The small number of sera tested from Varandha casts doubt on the significance of the substantial percentage of positives in that village when it is noted that the four positives are older adults. This appears similar to the distribution of immunity in Alibag and Murud, seaside localities where the positive occur in the older age group. If this type of virus infection is periodically epidemic, these findings reveal growing populations of susceptible non-immunes.

The observation of low or non-existent incidence of dengue I in several localities is at variance with the map makers on the other side of the world who have blanketed India as entirely a dengue endemic area. This is our first key to a West Nile active dengue absent area. No *Ædes ægypti* mosquitoes have yet been collected in the dengue negative localities.

TABLE XIII.

Neutralizing antibodies to West Nile virus in certain localities of Kolaba District.

	MAHAD					GOREGAON					MANGAON				
	—	Inc.	+	Total	Per cent +	—	Inc.	+	Total	Per cent +	—	Inc.	+	Total	Per cent +
0–4															
5–9	9			9	0	2			2	0					
10–14	11	1	7	19	37	14	2	4	20	20	4		4	8	50
15–19	13		12	25	48	6	3	5	14		5		6	11	55
20–29	3		3	6	50	1	1		2	0			3	3	100
30–39	4		2	6	33			1	1	100			3	3	100
40–49		1	1	2	50			3	3	100					
50+	1		3	4	75								2	2	100
Totals	41	2	28	71	40	23	6	13	42	31	9		18	27	67

	VARANDHA					INDAPUR AND WAVE DIVALI					KOLAD				
	—	Inc.	+	Total	Per cent +	—	Inc.	+	Total	Per cent +	—	Inc.	+	Total	Per ecnt +
0–4															
5–9						3		1	4	25	1			1	0
10–14	3			3	0	7	1		8	0	7			7	0
15–19	2			2	0	7			7	0					
20–29	3		1	4	25	6			6	0	2			2	
30–39	2		1	3	33	1			1	0			1	1	100
40–49												2		2	0
50+			2	2	100						1			1	0
Totals	10		4	14	29	24	1	1	26	4	11	2	1	14	7

TABLE XIII—(*Contd.*)

	BHIRA					ALIBAG					MURUD				
	—	Inc.	+	Total	Per cent +	—	Inc.	+	Total	Per cent +	—	Inc.	+	Total	Per cent +
0–4						0	0	0	0		0	0	0	0	
5–9	1			1	0	10	0	0	10	0	11	0	0	11	0
10–14						8	1	1	10	10	12	1	0	13	0
15–19	1			1	0	2	0	0	2	0	8	1	0	9	0
20–29	11	2		13	0	6	2	2	10	20	9	1	3	13	23
30–39	1	1	1	3	33	2	0	1	3	33	6	0	2	8	25
40–49	2			2	0	1	1	2	4	50	2	1	2	5	40
50+	1	1	1	3	33	0	0	2	2	100	0	0	3	3	100
Totals	17	4	2	23	9	29	4	8	41	20	48	4	10	62	16

TABLE XIV.

Neutralizing antibodies to Dengue I virus in certain localities of Kolaba District.

	MAHAD					VARANDHA					ALIBAG				
	—	Inc.	+	Total	Per-cent +	—	Inc.	+	Total	Per-cent +	—	Inc.	+	Total	Per-cent +
0–4															
5–9	1	1		2	0						8	2	0	10	0
10–14	11	2		13	0	3			3	0	5	4	0	9	0
15–19	23	1		24	0	2			2	0	1	1	1	3	25
20–29	5	1		6	0	4			4	0	3	4	3	10	30
30–39	4			4	0	3			3	0	1	1	1	3	33
40–49											1	0	2	3	66
50+	3	1		4	0		2		2	0	0	0	2	2	100
Totals	47	6		53	0	12	2		14	0	19	12	9	40	23

Such serological collections will be continued in our eighty mile square area, progressing systematically northward as far as Bassein in Thana District. This will, of course, take in the close confines of Santa Cruz Airport and Bombay Harbour which are potential receptor areas to yellow fever via ships, boats and airplanes which are continually arriving from East Africa, an endemic area of yellow fever. It has been theorized that dengue endemicity may be one active barrier to the introduction of yellow fever into eastern Asiatic areas which have remained free of the infection so far. It is, therefore, of some considerable importance to know the serological antibody status of people living in areas contiguous to a port receiving traffic from a yellow fever endemic area, as well as what the vector potential may be in case of an accidental introduction of the virus. This, therefore, is one of the important objectives of current studies in this specially selected region of long term virus investigations.

Field investigation routine.—Routine investigations were commenced at Mahad because of the proximity to different productive collecting localities and the large government dispensary with helpful co-operation of Dr. P. S. Babre and local practitioners and school officials. In Mahad early in August, the upper floor of a house containing three rooms and a veranda with tile floors was rented and equipped as a field station. Racks for mouse boxes were installed to enable field inoculation of serum of acutely febrile patients. The laboratory was also equipped with tables, microscope, centrifuge, kerosene refrigerator drawers, cupboards and other necessary items.

A routine programme was organized for field operations. Early each Monday morning one or two vehicles left the VRC, Poona, with the field team consisting of an MBBS Assistant Research Officer, Zoologist and field attendants and Entomologist and insect collectors. The A.R.O. each week took eighteen pregnant mice for provision of litters at the field station and on arrival began daily visits to clinics and dispensaries to obtain blood specimens from cases of acute febrile illness of no distinct diagnosis, after checking blood smears for absence of malarial parasites.

The entomological team undertook systematic collections of blood-sucking arthropods, primarily mosquitoes in and around human habitation. These arthropods were divided according to species for each locality and transported every other day by courier in a bus to the VRC where they were triturated and inoculated into mice for virus isolations. On Friday the team moved north for a night collection at Umbre near Khopoli and returned to Poona by Saturday noon with their latest collections and screened cages of inoculated mice.

The Zoologist and attendants collected birds and mammals of different localities for serum for antibody studies and preservation of brains and spleens in glycerine for later inoculation for virus isolation.

The routine program of investigation was centred at Mahad during August, September and October. The response of febrile patients reluctant to part with samples of blood for virus isolation attempts was disappointing. Consequently, human blood collection activities were transferred to Karjat and Khopoli, where a community project and specially interested practitioners, Drs. M. D. Bhatia and G. K. Gokhale, as well as Health Visitors, promise a better yield of febrile blood specimens, particularly from small children who are the best candidates because they are frequently infected with viruses to which they are not yet immune.

In August, September, October and November a total of 70 (Mahad 43, Mangaon 2, Khopoli 22, Karjat 3) sera collected from persons—mostly children—in an early acute phase of a febrile illness were inoculated IC into infant mice without yielding a strain of virus.

For this same period, entomological collections have continued on a routine basis, covering the same localities each week. Species and numbers collected are given in the Statistical Summary—Department of Entomology. Pools of most of these were inoculated into infant mice at the VRC for virus isolation as tabulated in the Statistical Summary—Department of Virology.

742 pools of arthropods collected between July 15 and November 30, 1955 yielded two strains of virus.

A pool (G1110) of 14 female *Culex vishnui* mosquitoes, collected in houses of Mangaon, on September 1, yielded the G1110 strain of virus. Neutralization testing of the strain against reference immune sera identified it as another strain of Sindbis virus.

A pool (G1424) of 32 female *Culex bitæniorhynchus* mosquitoes collected biting man in an outside station in a island of thick forest at Umbre, 8 miles south of Khopoli, on October 14, produced the G1424 strain of virus which is as yet unidentified. Its incubation period in infant mice is 3 to 5 days and in 3 weeks old mice 4 to 7 days. In eight mouse passage it consistently kills both infant and adult mice. It appears to withstand lyophilization without much loss of titre.

It is interesting to note that the strain behaves differently from any other agent under study in the VRC.

203 arthropod pools (Statistical Summary-Tissue Culture Section) were also inoculated into monkey kidney cell tissue culture tubes. No viruses were isolated although the cultures withstood the inoculum satisfactorily. 1,000 units of penicillin and 1 mg. streptomycin per c.c. is routinely added to arthropod suspensions before inoculation into mice, embryonated eggs or tissue culture.

Initial efforts in this long term field investigation area have already yielded considerable serological information of value and two strains of virus from arthropods. It was originally planned to maintain the studies on a year-round basis. However, present limitation in staff and transport equipment has resulted in drastic curtailment of investigation in this area at the present time because of diversion to work on another encephalitis problem, described in the next section of this report. Nevertheless, it s hoped that with an increase in staff and transport, study of the Kolaba-Poona-Thana District can be resumed in a routine capacity in the near future.

ARTHROPOD-BORNE VIRUS TYPE OF ENCEPHALITIS IN NORTH ARCOT DISTRICT OF MADRAS STATE:

A letter written on October fifteenth by Dr. John K. G. Webb, Professor of Pediatrics, Christian Medical College Hospital, Vellore, was received at the Virus Research Centre on October 17, stating that Dr. Webb had seen eight cases of what appeared to be a virus encephalitis on his ward since early September. It inquired whether we could give any help in identifying a possible virus etiology of the cases, and requested information about collecting material from patients and autopsies for virological examination.

There were some similarities to the Jamshedpur fever type of cases described previously in this report as being investigated for a viral etiolgy, but it was noted that the pleocytosis and increase in protein in the CSF were not compatible with the diagnosis of Jamshedpur fever. Detailed clinical information on the cases was requested by telegram and letter. Instructions were sent regarding collection of material for virological investigation.

On studying the case histories received a few days later, it was apparent that the course, general neurological involvement and sequelæ differed from the experience with Jamshedpur fever. When word of a new admission was received, it was decided to make preliminary investigations of the cases in Vellore and the Director departed by train on October 29 with mice for inoculation and equipment for serological collections.

Shortly after arrival in Vellore an 11 years old boy, who had been comatose on the Pediatrics Ward for a month died and came for postmortem examination. Brain and other organs of this case were inoculated into mice for virus isolation but as expected, because the brain material was obtained so long after onset, the mice showed no evidence of virus infection.

Half of this brain, in addition to brain sections from the first case which died early in September, was delivered for examination to Dr. C. G. S. Iyer, the ICMR Neuropathologist in Bombay.

Clinical cases.—Four other children on the ward and later admissions were observed as classical cases of virus encephalitis, with history of rapid onset of fever, headache, vomiting, convulsions and signs of severe cerebral and meningeal involvement. Most had stiff necks and were comatose with variable types of spastic paralysis, absent or hyperactive reflexes of varying distribution and positive Babinski reflexes on one or both sides. Blood count usually showed some leucocytosis and cerebrospinal fluid was clear, rarely under pressure, and usually with marked lymphocytic pleocytosis. Ages ranged from two to eleven years. None observed were older. A clinical evaluation of the problem will soon be reported by Dr. John K. G. Webb who has been responsible for their investigation.

Serological identification of etiology.—Convalescent specimens of differing vintage were obtained from the patients and, in addition, an acute early convalescent pair from a newly admitted case. Immediately on return to Poona, these were set up in a one dose neutralization test against Japanese B encephalitis (JBE) virus, along with more than eight other sera of family and village contacts of the patients.

All six convalescent sera neutralized the virus, demonstrating the 100 per cent prevalence of antibodies to an agent similar to or identical with JBE virus. This was strong circumstantial evidence of the type of etiological agent. As a check on the JBE results, all the sera in the first neutralization run were retested against Murray Valley Encephalitis (MVE) virus is known to be very closely related immunologically to JBE. The results of this test were almost identical with the first test against JBE virus, thereby confirming the original results and strengthening the validity of the evidence.

While the sera were in screening test against MVE, all the convalescent sera were tested against ten fold dilutions of both JBE and MVE viruses. The results, shown in Table XV, give evidence that the cases suffered from infection with Japanese B virus, not only because of a significant rise in titre in one case and an apparent rise in another pair, but also in the strength of neutralizing indices. The greater neutralizing capacity against Japanese B than Murray Valley virus may indicate greater specificity for JBE.

TABLE XV.

Titration of Japanese B and Murray Valley encephalitis viruses against convalescent sera of encephalitis cases in Vellore.

Case / Controls		Age/ sex	P number	Days after onset	Japanese B titre	NI	Murray Valley Titre	NI	
V1	Periakolandai	7/F	3080	30	$10^{-4.3}$	>2·5	$10^{-3.8}$	2·9	
4	Amsaveni	11/F	3087	40	$10^{-3.4}$	>3·4	$10^{-4.3}$	2·4	
7	Chandrasekran	5/M	3113	33	$10^{-2.6}$	>4·2	$10^{-2.8}$	3·9	
8	Muniratnam	8/M	3129	31	$10^{-2.6}$	>4·2	$10^{-3.4}$	3·3	
9	Paneer	4/M	3130	6	$10^{-4.5}$	>2·3	$10^{-4.6}$	2·1	
			3131	13	$10^{-2.3}$	>4·5	$10^{-3.5}$	3·2	<ed in
10	Jeyapalan	6/M	3146	9	$10^{-3.2}$	>3·6	$10^{-3.5}$	3·2	
			3132	10	$10^{-2.9}$	>3·9	$10^{-3.3}$	3·4	
				(36hours)					<ed in
KVS			3226	Survey<	$10^{-4.8}$	>2·0	$10^{-6.2}$	·5	
THW			2360	Vaccinated*	10^{-5}	>1·8	$10^{-5.0}$	1·7	
Rhesus	Monkey		R54	Immune	$10^{-5.6}$	>1·2	$10^{-5.6}$	1·1	
			BAPS	Control	$>10^{-6.8}$		$10^{-6.7}$		

* Vaccinated vs Jap B in 1946, 1952.
U Paired—show rise in titre

Additional information on the serological response of the patients and confirmation of the neutralization test titrations were obtained in complement-fixation tests with antigens to Japanese B and West Nile (WN) viruses, prepared by Dr. Jordi Casals in the Virus Laboratories of The Rockefeller Foundation in New York and delivered by air to Bombay at the end of November. These tests inaugurated routine complement fixation test facilities of the VRC. Table 16 gives CF results against JBE and WN antigens for sera collected from more than a dozen convalescent patients studied by the end of November.

TABLE XVI.

Complement-fixation tests of acute and convalescent sera of North Arcot District encephalitis cases against Japanese B and West Nile virus antigens.

Case ‡	Age/ sex	P number	Date of serum	Date of onset	Days PI	CF ANTIGEN	
						JBE	WN
2	9/F	3360	26–11–55	4–9–55	80	<16	<ed 16
V 1	7/F	3080	2–11–55	1–10–55	30±	32	<ed 16
7	5/M	3113	31–10–55	30–9–55	31—	64	<ed 16
		3351	20–11–55		51	64	<ed 16
		3352	26–11–55		57	128	<ed 16
8	8/M	3129	31–10–55	2–10–55	29	>256	32
		3346	20–11–55		50	>256	16
		3347	26–11–55		56	>256	32
4	11/F	3087	1–11–55	19–9–55	40	32	4
		3350	21–11–55		60	32	4
9	4/M	3130	24–10–55	19–10–55	6	<4	
		3131	31–10–55		13	64	8
		3307	20–11–55		34	64	4
		3308	24–11–55		38	64	4
10	6/M	3146	30–10–55	21–10–55	9		
		3132	31–10–55		10	>128	8
		3344	20–11–55		30	>128	128
		3345	26–11–55		36	>128	64
11	6/M	3348	9–11–55	28–10–55	12	32	<ed 4
		3258	20–11–55		23	64	4
		3349	26–11–55		29	64	4
12	6/M	3295	10–11–55	5–11–55	6	<4	<ed 4
		3296	20–11–55		16	64	<ed 4
		3297	26–11–55		22	32	4
13	7/F	3356	24–11–55	20–11–55	4	4	4
		3357	26–11–55		6	4	<ed 4
R1	/M	3362	26–11–55		9	<4	<ed 4
R2	/M	3364	26–11–55		20	<16	<ed 16

This serological evidence fairly definitely established that the encephalitis cases observed and investigated in Vellore and North Arcot District of Madras suffered from an arthropod-borne neurotropic virus infection caused by a virus similar to or identical with Japanese B.

Epidemiological investigations.—On the first trip to Vellore, visits were made to village homes of seven cases which had been observed on the Pediatrics Ward of the hospital. A list of home addresses of the patients was obtained by Dr. M. D. Graham and visits were arranged in two days with the help of Dr. H. Rao, Director of Rural Health Survey and Dr. Le Roy Allen, Professor of Preventive and Social Medicine in the College. These were made to obtain epidemiological observations and information as well as to collect sera from family contacts, neighbours and school-mates of the cases. All patients' homes were in rural villages varying from two to twenty-six miles distance from Vellore. Five more village homes of cases were visited on a second trip to the area later in November, and some pertinent observations of all case origins have been tabulated in Table XVII.

These features demonstrate a marked similarity in environmental relationships of the homes in which cases were apparently exposed. Homes of the cases are usually mud plastered brick structures with thatched or tile roofs, many constructed around a central compound open to the sky. Domestic animals, consisting most commonly of chickens and cattle, are tethered in these compounds or on front porches and occasionally in adjoining structures or in the backyard. The houses are either adjacent to or, at most, within a hundred yards of open rice fields, which are breeding places for *Culex* (particularly *C. vishnui*) mosquitoes, and cane and cholam fields, which are favourite daytime resting harborage for *Culex* mosquitoes. *Anopheline* mosquitoes are not uncommonly found resting inside patients' houses. The village localities where these conditions occur and from which encephalitis patients have come are widely scattered throughout North Arcot and neighbouring Districts, the most distant observed to-date being about sixty miles from Vellore. This wide distribution of occurrence of cases in a district more than 4,400 square miles in area is an epidemiological characteristic of an arthropod-borne virus encephalitis epidemic.

During these visits, information about two additional cases, one fatal and the other with serious post-encephalitic paralysis a month after onset was obtained in two villages from which fatal hospital cases had come. This latter, case VI, provided a convalescent serum specimen with which diagnosis was confirmed. These observations indicate the possibility that there have been many rural cases which have never reached the hospital. An attempt is being made to obtain information on this aspect of the problem to get a more satisfactory idea of the possible attack rate.

Most of the sera of close family contacts also gave positive serological tests. Evidence of this family exposure is particularly striking in the relations of case 7 as shown in Table XVIII. This family lived in a farm house at least a mile from the nearest village. The house was surrounded by the fields of cane, cholam and well watered rice and man biting mosquitoes were abundant. There was a lesser, but significant incidence of positives in village neighbours of other cases and a still smaller, but substantial, number of positives among young school-mates of cases, where sera could be collected.

Rainfall observations.—An unusual, continuous and considerable rainfall during July and August 1955 was repeatedly reported by residents of the rural villages where cases have originated. Data obtained from official reports in various taluks of the district confirm this observation. This earlier continuous rainfall than previously experienced may be a key to the occurrence of an abnormally enlarged mosquito population this year, complained of without exception by everyone encountered in the rural areas of North Arcot District.

TABLE XVII.

Environmental features of village homes of virus encephalitis cases in North Arcot (Madras) and Chittoor (Andhra) Districts.

Case	Name	Age/ sex	Location	Distance/ direction	Adjacent to fields	Distance from fields	Open rice	Close stable	*Culex vishnui*
1	Pachamma	2/F	Ariyapadi	19 m SE	—	100 yd.	+	Porch	
2	Yeshoda	9/F	Venatapuram	5 m NE	+	25 ft.	+	Backyard	+
3	Pichande	5/M	Sandavasal	26 m S	—	50 yd.	+	+	+
V1	Periakolandai	7/F	Sandavasal	26 m S	+	*	+	+	+
4	Amsaveni	11/F	Sathuperi	2 m W	—	100 ft.	—	Compound	+
5	Raghupathy	8/M	Gururajpet	50 m NE	—	100 yd.	+	—	+
6	Govindaswamy	11/M	Peria Kalampadi	35 m S	—	100 yd.	+	Porch	
7	Chandrasekran	5/M	Kammavanpettai	15 m SE	+	†	+	Porch and compound	+
8	Munirathnam	8/M	Melkodungalur or Eyipakkam	60 m SE	+	*	+	Compound	+
9	Paneerselvan	4/M	Sathiram Puthur	30 m E	+	*	+	Compound	+
10	Jeyapalan	6/M	Seelery	12 m W	+	†	+	Compound	+
11	Govindswamy	6/M	Minnal	50 m NE	+	100 ft.	—	Porch	+
12	Govindswamy B	6/M	Bapnapalli	12 m N	+	50 ft.	+	Compound	+

Distances are approximate (sight estimates)

* House adjacent to fields.

† House surrounded by fields.

TABLE XVIII.

Neutralization and complement-fixation test results for family contacts and associates of case 7.

Date of visit : 1-11-1955.

P number	Name/address	Age/sex	Category	Jap. B	MVE	CF antigen	
						JBE	WN
3113	Chandrasekaran Velayudham	5/M	Convalescent patient.	+	+		
3114	Elandan Munusamy	56/M	Father	+	+		
3115	Muniamma	35/F	Mother	+	+	4	8
3116	Munusamy	23/M	Brother	+	+	> 16	8
3117	Murugesan	14/M	Brother	—	—		
3118	Pushpa	18/F	Sister	—	—		
3119	Dhanam	8/F	Sister	+	+		
3120	Poongavanam	65/F	Grandmother	+	+		
3121	Chinnayya Munusamy	65/M	Uncle	+	+		
3122	Chinnamma (1st wife of Chinnyya)	55/F	Aunt	+	+	> 16	8
3123	Visalam (2nd wife of Chinnayya)	35/F	Aunt	+	+		
3124	Bankiam	15/F	Cousin	+	+		
3125	Velu	10/M	Cousin	+	+	8	> 16
3126	Ayyamma	12/F	Cousin	+	+	4	4

Long term field investigation.—The field team, consisting of entomologist, zoologist, insect collector, two zoological field attendants and a driver in a Chevrolet Carryall of the VRC, containing equipment for field collections, departed from Poona on November 17, for the 700 mile trip by road to Vellore. They arrived on November 19, the same date that the Director and a senior Laboratory Technician of the VRC arrived by train with laboratory equipment and mice for installation in laboratory and animal house facilities generously made available from limited space in the hospital by Dr. Ruth Meyers, Professor of Bacteriology, and Dr. Gordon Hadley, Acting Professor of Pathology and administrative authorities of Christian Medical College.

In addition, visits were made to hospitals in other towns of the district to obtain information about possible additional cases which might have been admitted elsewhere. Dr. Savarirayan of the hospital at Ranipet reported six or seven cases which had been seen in that hospital in the past two months and arrangements were made to obtain details on these cases for later visits and convalescent serum collection. One hospital was visited where two cases had been treated before coming to our notice. The two physicians in attendance, however, denied any knowledge of such patients as we described occurring this year. This emphasized the difficulty which will be encountered in attempting to obtain any indication of the clinical attack rate on the basis of such information collection.

Arrangements were made with Dr. A. Chcokalingam, Health Officer of North Arcot District to circularize medical and sanitary personnel under his jurisdiction concerning reporting of encephalitis or suspicious cases. This will be followed up with visits to the twenty-five Maternity and Child Welfare Centres of the District.

Entomological observations.—Intensive entomological collection has been undertaken in the homes, neighbouring buildings and stables and fields adjacent to the village homes of cases. *Culex vishnui* has been found in great abundance in all of the villages visited and in all of these types of habitats. *C. vishnui* larvæ been found in the well watered rice fields. *C. fatigans* (*quinquefasciatus*) has been found in numbers frequently as has *C. bitæniorhynchus* but not consistently nor in a significant fraction of the numbers in which *C. vishnui* has been encountered and collected.

With the employment of three additional novice insect collectors the daily collection of *C. vishnui* ranged from a thousand to 1,500 females. Most of these are caught by hand from resting locations, but the collection is augmented by light trap, chicken baited trap, and human and animal biting catches in two locations. A few *C. gelidus* have been found but no *C. tritæniorhynchus*.

Anopheles fluviatilis, *A. jamesi* and other anophelines have been found resting inside houses in lesser numbers per species, but in overall abundance. These too have been collected and pooled for inoculations into infant mice for attempts to isolate virus from arthropod vectors.

C. vishnui is closely related to *C. tritæniorhynchus*, the Japanese B vector in Japan and to *C. gelidus* which has been incriminated in Malaya. In habits it is similar to *C. tritæniorhynchus* and *C. tarsalis*, the vector of western equine and St. Louis encephalitis in western U.S.A., in being an open wet field breeder and having a wide spectrum of hosts for blood meals. *C. vishnui* in the Vellore area has been collected only not in houses, stable, and field resting habitats, but also biting man, cattle and other domestic quandrupeds as well as from a chicken baited mosquito trap.

There are records of isolation of Japanese B virus from *Anopheles hyrcanus* in Japan, so anophelines cannot be ruled out as vectors. However, the great abundance of *C. vishnui* consistently found its variety of blood meal hosts, which may be involved in the epidemiology of arthropod borne encephalitis, makes it the most highly suspect as a vector of the virus encephalitis now being investigated in North Arcot District of Madras.

Zoological observations.—Wild birds have been incriminated elsewhere as circulating virus reservoirs of arthropod-borne neurotropic viruses of the Japanese B-Murray Valley-West Nile-St. Louis group. For this reason collection of the common wild birds of the village environments has been undertaken immediately for attempts to isolate virus from bird spleens by inoculation of spleen suspensions intracerebrally into infant mice as litters become available. Later, similar examination will be made of brain specimens now being tested for neutralizing antibodies to Japanese B virus to determine whether they may have been important hosts to this virus.

The common wild birds appear to be similar in all localities where case origins have been studied. They are in probable order of abundance; House sparrow, *Passer domesticus*, Common Mynah, *Acridotheres tristis*, House crow, *Corvus splendens*, Jungle crow, *Corvus macrorhynchus*, Rose-ringed parakeet, *Psittacula krameri*, Paddy bird, *Ardeola grayi*, and cattle egret, *Bubulcus ibis*.

There are many other smaller species in significant numbers. But it is thought that the species being collected should give an intimation of the possible rôle of wild birds in the present problem as an indicator towards future consideration of other species. Significant numbers of specimens have been collected from all above mentioned species except the House sparrow, and are being or about to be tested as mice become available.

Possible vertebrate reservoirs.—Eight cattle sera, three sera of flying foxes (*Pteropus gigantius*), one serum from a Black-naped hare (*Lepus cunculus*), and thirty-two sera collected from eight avian species collected in North Arcot District as listed below, were neutralization tested against Japanese B virus. None showed positive evidence of specific antibodies. This can be considered a possible indication that vertebrates in close association with cases in this area do not play the expected rôle as vertebrate reservoirs of the virus for occasional mosquito transmission to man. This is particularly remarkable considering the evidence of massive exposure (Table XVIII) in the family of case 7 on whose farm many of the sera tested were collected.

AVIAN SERA TESTED

Swallow, *Hirundo rustico*	1
Jungle crow, *Corvus macrorhynchus*	4
House crow, *Corvus splendens*	2
Common mynah, *Acridotheres tristis*	2
Rose-ringed parakeet, *Psittacula krameri*	3
Paddy bird, *Ardeola grayi*	10
Cattle egret	5
Chicken	5
Total	32

This is one of the many unusual observations which will doubtless be made concerning the epidemiology of this newly recognized human disease problem in India. While the prevention and control of this infection awaits thorough investigation of the problem with accumulation of data on all aspects of its natural history, it is a challenge to the public health and medical scientists of a populated area where there is indication that it will be a serious threat to human health in the future. It appears that southeastern India is such an area.

OTHER STUDIES OF THE VRC.

Rabies.—(*a*) Four glycerinated guinea-pig brains representing four strains isolated from horse brains between 1940 and 1946 were received at the Virus Research Centre from the Indian Veterinary Research Institute, Mukteshwar-Kumaon, U.P. These were believed to be strains of Eastern Equine Encephalitis and were maintained in guinea-pigs by intracerebral or subdural inoculation. Their passage levels were unknown.

Each of these was passed into mice and the fresh mouse brain suspension of the paralyzed mice was run against rabies hyper immune serum. The serum neutralized each strain by more than 3·0 logs LD_{50}.

It was concluded that the strains were of rabies virus..

(*b*) A strain isolated at the Armed Forces Medical College, Poona, from the brain of a 15-year old male of Secunderabad with a post-mortem diagnosis of encephalitis, was identified as rabies virus.

(*c*) An eight year old child, who had a history of dogbite, and had received 15 doses of antirabic vaccine, died at Sassoon Hospital, Poona, after an illness clincially recognized as encephalitis. The autopsy brain material of this child yielded a virus strain which was immunologically identified as rabies virus.

Chicken strains.—A locally purchased chicken, which was used for feeding presumably non-infective mosquitœs in the entomology laboratory died on October 17, 1955 and two strains of virus, F811 and F812 were isolated from the brain and spleen respectively. These agents are pathogenic only for infant mice and are being studied for identification.

STATISTICAL SUMMARY.

DEPARTMENT OF VIROLOGY.

(A) *Animal Virology Section.*

1. Summary of specimens tested for the presence of virus, mostly by inoculation of the test material into infant mice by the intracerebral route—October 1954 through November, 1955.

Human material:

Blood serum	88
Brain tissue	6
C.S.F.	8
Miscellaneous	5
Stools	65
Sub total	172

Lower mammal material:

Mouse	4
Cotton rat	1
Goat	3
Dog	1
Cat	1
Hamster	2
Sub total	12

Bird material:

Crow, common house	21
Crow, jungle	9
Koel	9
Domestic pigeon	5
Domestic chick	7
Rose-Ringed parakeet	1
Paddy bird	10
Common mynah	3
Cattle egret	6
Sub total	71

Arthropod material:

	Number of pools	Number of arthropods
Mosquitœs.		
Ædes vexans	5	37
Ædes albopictus	31	1,030
Ædes w-albus	13	303
Ædes mixed spp.	18	308
Ædes gubernatoris	6	64
Ædes vittatus	3	56
Ædes dissimilas	1	6
Ædes cogilli	1	4
Ædes thomsoni	1	13
Armigeres obturbans	177	7,162
Armigeres digitatus	2	8
Culex bitæniorhynchus	58	998
Culex fatigans	118	4,186
Culex gelidus	45	1,647
Culex vishnui	164	7,302
Culex whitmorei	3	81
Culex fuscanus	1	4
Culex khazani	9	144
Culex pluvialis	4	57
Culex malayi	1	12
Culex mixed spp.	39	551
Culex sitiens	1	5
Culex minutissimus	2	24
Culex pallidothorax	7	88
Culex lophoceratomyia	3	37
Mansonia crassipes	1	6
Manosnia uniformis	1	5
Uranotænia campestris	2	22

	Number of pools	Number of arthropods
Uranotænia maculipleura	1	34
Uranotænia recondita	3	80
Uranotænia stricklandi	3	40
Uranotænia mixed spp.	6	95
Anopheles culicifacies	56	1,633
Anopheles subpictus	48	1,720
Anopheles tessellatus	9	106
Anopheles moghulensis	3	59
Anopheles hyrcanus	1	8
Anopheles barbirostris	11	514
Anopheles vagus	19	410
Anopheles pallidus	16	358
Anopheles jamesi	29	773
Anopheles mixed spp.	56	1,374
Tripteroides aranoides	3	56
Heizmannia viridis	1	20
Mosquito sp. (undetermined)	1	24
Sub total	983	31,464
Sandflies :		
Phlebotomus argentipes	28	2,596
Phlebotomus papatassi	14	564
Sub total	42	3,160
Mites :		
Bdellonyssus bursa	11	15,250
Lælaptidæ	2	365
Gen. et sp. undetermined	1	17
Sub total	14	15,632
Fleas :		
Ctenocephalides felis	1	58
Gen. et sp. undetermined	2	19
Sub total	3	77
Ticks :		
Rhipicephalus sanguineus	4	52
Rhipicephalus hæmaphysaloides	12	167
Rhipicephalus sp. undetermined	1	20
Hæmaphysalis probably *parva*	7	77
Hæmaphysalis parva	18	208
Hæmaphysalis bispinonsa	1	8
Hyalomma ægyptium	9	88
Hyalomma species—undetermined	1	3
Hyalomma hussani	1	1
Boophilus australis	6	21
Sub total	60	645

Miscellaneous groups:	Number of pools	Number of arthropods
Mallophaga (Lica)	1	67
Hippoboscidæ (flies)	2	24
Culicoides anopheles (biting midgets)	2	97
Culicoides species—undetermined (Biting midgets)	10	1,497
Sub total	15	1,685
Totals	1,117	52,663

Grand total of specimens tested for virus 1,372.

2. Summary of specimens tested for presence of virus in chick embryos between 25-10-55 and 30-11-55 by chorioallantoic or yolk sac route.

Arthropod material:

Locality	Number of arthropod pools
Kolaba District	61
North Arcot District	8
	69

Total number of chick embryos used for isolation and passages 532

(B) *Tissue Culture Section.*

Summary of specimens tested for the presence of virus by inoculation of the test material in monkey kidney cell tissue culture—June 1955, through November 1955.

Human material:	
Brain Tissue	2
C.S.F.	2
Miscellaneous	3
Stools	65
Sub total	72

Arthropod material:	Number of pools	Number of arthropods
Ædes albopictus	11	418
Ædes w-albus	5	111
Ædes mixeds pp.	9	128
Ædes gubernatoris	2	10
Ædes thomsoni	1	13

	Number of pools	Number of arthropods
Armigeres obturbans	67	3,026
Armigeres digitatus	1	4
Culex bitæniorhynchus	1	32
Culex fatigans	4	117
Culex gelidus	14	363
Culex vishnui	36	1,187
Culex spp.	2	22
Mansonia uniformis	1	5
Anopheles culicifacies	8	154
Anopheles subpictus	6	132
Anopheles tessellatus	4	51
Anopheles barbirostris	2	13
Anopheles vagus	4	83
Anopheles pallidus	5	142
Anopheles jamesi	6	144
Anopheles mixed spp.	11	395
Sub total	200	6,550
Sandfiies :		
Phlebotomus argentipes	3	392
Total	203	6,942
Grand total of specimens tested for virus	275	

DEPARTMENT OF FIELD EPIDEMIOLOGY.

Summary of human specimens collected for serological and virus isolation studies—October 1954 *through November* 1955.

1. *Human brain.*	
Poona	3
Secunderabad ...	1
Delhi	1
Vellore	1
	6
2. *C.S.F.*	
Poona	3
Secunderabad ...	1
Vellore	3
Nanipet	2
	9
3. *Stools.*	
Poona	4
Khadakwasla	14
Delhi	29
Vellore	1
	48

4. *Viscera.*

Delhi	1
Vellore	1
	2

5. *Human sera.*

Locality		CATEGORY			
	Acute	Convalescent	Contact	Survey	Total
1. Delhi	22	16	3		47*
Sub total ...	22	16	3	0	47
2. *East Bengal.*					
Calcutta ...	...	...	...	50	50
Calcutta filariasis ...	...	...	...	18	18
Diamond Harbour ...		...	...	58	58
Maricha	...	...	...	51	51
Sub total ...	0	0	0	177	177
3. *Poona.*					
V.R.C. ...	5	...	...	32	37
Poona City ...	9	1	...	77	87
Khandakwasla ...	3	...	...	...	3
Sub total ...	17	1	0	109	127
4. *Kolaba District—Bombay State.*					
Mahad ...	43	9	...	88	140
Mangaon ...	2	...	...	45	47
Goregaon ...	...	...	...	47	47
Varandha ...	...	...	...	16	16
Manjri	...	...	...	2	2
Poladpur ...	...	...	...	79	79
Indapur-Wavedivali ...	...	...	...	45	45
Kolad	...	...	...	19	19
Bhira	...	...	...	51	51
Pen	...	...	...	45	45
Jambivali-Savarse ...	...	...	...	23	23
Umbre	...	...	...	8	8
Khapoli	22	...	...	...	22
Karjat	3	...	...	26	29
Alibag	...	...	...	46	46
Murud	...	...	...	67	67
Sub total ...	70	9	...	607	686

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* No information available on 6 serum specimens from Delhi.

5. *North Arcot District—Madras State.*

Ariyapadi ...	...	...	10	...	10
Periakalampadi ...	...	...	3	...	3
Sandavasal ...	...	1	18	...	19
Sathuperi ...	...	1	28	...	29
Kammavanpettai ...	...	...	13	...	13
Venkatapuram ...	...	1	2	...	3
Seelery		...	11	...	11
Minnal	...	...	3	...	3
Gururajpet ...	...	...	33	...	33
Dakshana Brahmapalli	...	...	9	...	9
Kondakuppam ...	...	...	4	...	4
Sathiramputhur ...	...	...	9	...	9
Eyipakkam ...	...	...	13	...	13
Melkodungalur ...	...	...	9	...	9
Ranipet Hospital ...	1	1	...	...	2
Christian Medical College Hospital, Vellore	6	25	5	1	37
Sub total ...	7	29	170	1	207
Grand total ...	116	55	173	894	1,244

DEPARTMENT OF ZOOLOGY.

Number of mammals collected.

	Dead	Alive	Total
Poona District:			
Funambulus pennanti	0	11	11
Funambulus sublineatus ...	0	11	11
Tetera indica	0	2	2
Bandicota bengalensis	0	2	2
Bandicota malabarica	0	1	1
Rattus rattus	0	4	4
Millardia meltada	0	6	6
Sub total	0	37	37
Kolaba District:			
Suncus murinus	0	1	1
Scotophilus timminickii	0	1	1
Funambulus sublineatus ...	2	0	2
Rattus rattus	0	4	4
Rattus blanfordu	0	2	2
Colunda ellioti	0	2	2
Sub total	2	10	12

	Dead	Alive	Total
North Arcot District:			
Pteropus giganticus	3	0	3
Lepus cuniculus	1	0	1
Sub total	4	0	4
Grand total	6	47	53

Mammal bloods and other tissues collected.

	Blood	Brain	Spleen	Sal. gland
Poona District:				
Funambulus pennanti ...	10	0	0	0
Funambulus sublineatus	8	0	0	0
Tetera indica ...	2	0	0	0
Bandicota bengalensis ...	1	0	0	0
Bandicota malabarica ...	1	0	0	0
Rattus rattus ...	4	0	0	0
Millardia meltada ...	5	0	0	0
Goats	25	0	0	0
Sub total ...	56	0	0	0
Kolaba District:				
Rattus rattus ...	4	0	0	0
Rattus blanfordii ...	1	0	0	0
Golunda elliottii ...	2	0	0	0
Goats ...	2	0	0	0
Sub total ...	9	0	0	0
North Arcot District:				
Pteropus giganticus ...	3	3	3	3
Lepus cuniculus ...	1	0	0	0
Cows	8	0	0	0
Sub total ...	12	3	3	3
Grand total ...	77	3	3	3

Number of birds collected.

	Dead	Alive	Total
Calcutta District:			
Corvus macrorynchos	15	0	15
Corvus splendens	51	0	51
Sub total	66	0	66

	Dead	Alive	Total
Poona District:			
Corvus macrorynchos	21	0	21
Corvus splendens	10	0	10
Eudynamis scolopaceuos	16	0	16
Sub total	47	0	47
Kolaba District:			
Corvus macrorhynchos	20	0	20
Corvus splendens	52	0	52
Maclolophus xanthogenys	1	0	1
Turdoides somervillii	3	0	3
Ægithina tiphia	3	0	3
Chloropsis Jerdonii	2	0	2
Molpastes cafer	2	0	2
Octocompsa Jocosa	1	0	1
Saxicola caprata	1	0	1
Phœnicurus ochruros	1	0	1
Saxicoloides fulicata	1	0	1
Copsychus saularis	1	0	1
Hypothymis azurea	1	0	1
Lanius schach	2	0	2
Tephrodornis pondicerianus	1	0	1
Pericrocotus perigrinus	2	0	2
Dicrurus macrocercus	4	0	4
Orthotomus sutorius	1	0	1
Prinia socialis	1	0	1
Oriolus oriolus	1	0	1
Oriolus xanthornus	2	0	2
Sturnia malabarica	1	0	1
Acridotheres tristis	2	0	2
Ploceus philippinus	1	0	1
Passer domasticus	2	0	2
Riparia concolor	2	0	2
Hirundo smittii	1	0	1
Hirundo daurica	1	0	1
Motacilla alba	1	0	1
Motacilla flava	1	0	1
Motacilla madraspatensis	1	0	1
Galerida cristata	3	0	3
Eremopteryx grisea	1	0	1
Cinnyris asiaticus	1	0	1
Cinnyris zeylonicus	1	0	1
Dicæum erythrorhynchos	1	0	1
Dryobates mahrattensis	1	0	1
Micropternus brachyurus	1	0	1
Xantholæma hæmacephala	2	0	2
Coracias benghalensis	1	0	1
Merops orientalis	2	0	2
Alcedo atthis	2	0	2

	Dead	Alive	Total
Halcyon smyrnensis	1	0	1
Dichoceros bicornis	2	0	2
Hydrocissa sp.	1	0	1
Tockus birostris	1	0	1
Upupa epops	1	0	1
Hierococcyx virus	1	0	1
Eudynamis scolopaceus	2	0	2
Centropus sinensis	2	0	2
Psittacula cyanocephala	1	0	1
Corryllis vernalis	1	0	1
Strix ocellatum	1	0	1
Ketupa zeylonensis	1	0	1
Pseudogyps Bengalensis	1	0	1
Spizaetus cirrhatus	1	0	1
Haliaster indus	1	0	1
Milvus migrans	1	0	1
Elanus cærulens	1	0	1
Circus macrourus	1	0	1
Aster badius	1	0	1
Falco juggar	1	0	1
Crocopus phœnicopterus	3	0	3
Columba livia	3	0	3
Streptopelia chinensis	5	0	5
Streptopelia senegalensis	1	0	1
Perdicula asiatica	0	3	3
Turnix sylvatica	1	0	1
Amavrornis phœnicura	1	0	1
Lobivanellus indicus	2	0	2
Tringa hypoleucos	1	0	1
Phalacrocorax niger	1	0	1
Anastomus ositans	1	0	1
Egretta intermedia	23	0	23
Ardeola grayi	1	0	1
Nycticorax nycticorax	1	0	1
Sub total	199	3	202
North Arcot District:			
Corvus macrorhynchos	11	0	11
Corvus splendens	72	0	72
Acridotheres tristis	28	0	28
Psittacula krameri	29	0	29
Hirundo rustica	10	0	10
Ardeola grayi	44	0	44
Bubulcus ibis	6	0	6
Sub total	200	0	200

	Dead	Alive	Total
Chittoor District:			
Corvus macrorhynchos	5	0	5
Acridotheres tristis	6	0	6
Psittacula krameri	2	0	2
Ardeola grayi	4	0	4
Sub total	17	0	17
Grand total	529	3	532

Bird bloods and tissues collected.	Blood	Brain	Spleen
Calcutta District:			
Corvus machrorhynchos	15	0	0
Corvus splendens	51	0	0
Sub total	66	0	0
Poona District:			
Corvus macrorhynchos	21	0	0
Corvus splendens	10	0	0
Endynamis scolopaceus	16	0	0
Sub total	47	0	0
Kolaba District:			
Corvus macrorhynchos	18	19	19
Corvus splendens	47	52	52
Turdoides somervilli	0	0	1
Ægithina tiphia	0	0	3
Chloropsis Jerdonii	0	0	2
Phœnicurus ochrurus	0	0	1
Saxicoloides fulicata	0	0	1
Lanius schach	0	0	1
Tephrodornis pondicerianus	0	0	1
Perecrocotus peregrinus	0	0	1
Dicrurus macrocereus	0	0	2
Oriolus oriolus	0	0	1
Acridotheres tristis	0	0	1
Ploceus philippinus	0	0	1
Passer domasticus	0	0	2
Motacilla madraspatensis	0	0	1
Galerida cristata	0	0	3
Xantholaema hæmacephala	0	0	1
Haleyon smyrnensis	0	0	1
Dichoceros bicornis	0	0	2
Hydrocissa sp.	0	0	1
Upupa epops	0	0	1
Hierococcyx varius	0	0	1
Endynamis scolopaceus	1	0	2
Centropus sinensis	0	0	1

	Blood	Brain	Spleen
Strix ocellatum	1	0	0
Ketupa zeylonensis	0	1	1
Pseudogyps bengalensis	0	0	1
Spizætus cirrhatus	0	0	1
Milvus gouinda	0	0	1
Crocopus phœnicopterus	0	0	3
Columba livia	0	0	3
Streptopelia	1	2	2
Phalacrocorax niger	0	0	1
Anastomus ositans	3	0	0
Egretta intermedia	19	23	23
Sub total	90	97	139
North Arcot District:			
Corvus macrorhynchos	11	11	11
Corvus splendens	65	72	72
Acridotheres tristis	24	28	28
Psittacula krameri	26	29	29
Hirundo rustica	1	10	9
Ardeola grayi	40	44	44
Bubulcus ibis	6	6	6
Chicken	18	0	0
Sub total	191	200	199
Chittoor District:			
Corvus macrorhynchos	5	5	5
Acridotheres tristis	6	6	6
Psittacula krameri	2	2	2
Ardeola grayi	4	4	4
Sub total	17	17	17
Grand total	411	314	355

2. Rabies inquiry under the Director, Central Research Institute, Kasauli.

I. ANTIBIOTICS IN THE TREATMENT OF RABIES

The prevention of fixed virus rabies in experimental animals by the parenteral administration of some of the newer antibiotics was tried. White mice, weighing 11 g. to 13 g., were used for the tests. The challenge virus employed for the purpose was the C.V.S. (challenge virus standard) strain of fixed virus given intramuscularly, into the thigh, an hour before commencement of treatment. The infecting dose consisted of 0·05 c.c. of a 10^{-3} dilution of the challenge virus. The results are shown in Table I.

TABLE I

Drug	Details of treatment	RESULTS:
		Death from rabies/total
Polymyxin B	0·01 mg. I.P. daily for 6 days	3/5
Viomycin	0·01 mg. I.P. daily for 6 days	5/5
Neomycin	100 units I.P. daily for 6 days	5/5
Achromycin	2 mg. I.P. daily for 6 days	4/4
B. Subtilis broth filtrate (corn).	0·25 c.c. I.P. on 1st day 0·75 c.c. I.P. on next 5 days	4/4
Untreated controls	10^{-3} C.V.S. strain I.M.	4/4
	10^{-4} ,, ,, ,,	2/4
	10^{-5} ,, ,, ,,	0/4

Comments.—None of the antibiotics under test showed any saving effect except that there was an indication that Polymyxin B. might possibly be of some value. The experiment was repeated using larger doses of this antibiotic and the same infecting dose of fixed virus. The results obtained are shown in Table II:—

TABLE II.

Drug	Dose, mg.	Route	Number of injections	RESULTS:
				Deaths/total
Polymyxin B.	0·05	I.P.	6	5/5
,,	0·1	,,	6	5/5
,,	0·2	,,	6	5/5
Untreated controls	...	...	...	4/5

Larger doses of Polymyxin B were found to be lethal to mice. Several others of the newer antibiotics have been obtained and are under tests.

II. PROTECTIVE VALUE OF THE SUPERNATANT FRACTION OF SEMPLE'S ANTIRABIC VACCINE.

Results of work on supernatant vaccine, prepared by centrifuging the brain emulsion of fixed virus infected sheep in an International centrifuge, has been communicated in early reports. The use of the Sharple's supercentrifuge for the preparation of the supernatant vaccine was investigated during the year.

Experiment I.—Semple's 5 per cent phenolized sheep brain vaccine in distilled water was prepared according to the method in routine use. Part of the vaccine was centrifuged at 25,000 r.p.m. using a separator bowl, fine jet and a rate of flow of 1 litre in 10 minutes, and the supernatant fluid collected.

The comparative total solids in the original and the centrifuged vaccine were as follows:—

	Total solids W/V
Whole brain vaccine ...	1·0 per cent
Supernatant ,, ...	0·5 per cent

After sterility and virus inactivation tests, comparative potency tests were carried out on both the supernatant vaccine so obtained and the original whole brain vaccine. Guinea-pig were used as experimental animals. They were challenged intracerebrally with fixed virus (C.V.S. strain) 10 days after the last injection of vaccine. The results obtained are shown in Table III:—

TABLE III.

Group	Treatment	Dilution of challenge: virus (C.V.S. strain):							LD_{50} of virus	Protection Log. of vaccine
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}		
I.	6×1 c.c. of 5 per cent whole brain vaccine intraperitoneally ...	3/7	3/7	3/6	3/6	1/6	...	...	3·2	4·3
II.	6×1 c.c. of 5 per cent supernatant vaccine intraperitoneally ...	3/6	1/6	3/6	2/6	3/6	...	...	3·3	4·2
III.	Untreated controls ...	...	...	...	...	6/6	5/6	1/5	7·5	...

Comments.—The protection afforded by both vaccines is almost identical.

Experimental II.—The experiment was repeated using another freshly prepared vaccine. The results obtained are given in Table IV:—

TABLE IV.

Group	Treatment	Dilution of C.V.S. strain fixed virus:							LD_{50} of virus	Protection Log. of vaccine
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}		
I.	6×1 c.c. of 5 per cent whole brain vaccine intraperitoneally ...	4/6	5/6	3/6	5/6	2/6	...	...	5·1	2·4
II.	6×1 c.c. of 5 per cent supernatant vaccine, intraperitoneally ...	5/5	4/6	5/6	2/6	3/6	...	...	4·8	2·7
III.	Untreated controls ...	...	...	...	...	6/6	6/6	0/6	7·5	...

Comments.—Here again the protection afforded by both vaccines is almost identical.

Experiment III.—Effect of re-centrifugation of antirabic vaccine on potency.—The experiment was once again repeated using another lot of freshly prepared antirabic vaccine. On this occasion the supernatant vaccine was given a second run through the Sharple's Centrifuge at 25,000 r.p.m. in order to determine the effect of further purification of the 1st centrifugate, on its potency. The results are show in Table V:—

TABLE V.

Group	Treatment	DILUTION OF FIXED VIRUS CHALLENGE (C.V.S. STRAIN):							LD_{50} of virus	Protection Log. of vaccine
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}		
I.	6 × 1 c.c. of 5 per cent whole brain vaccine intraperitoneally ...	3/7	3/5	1/6	6/6	1/6	...	...	3·3	4·94
II.	6 × 1 c.c. of supernatant second centrifugate, intraperitoneally ...	4/7	5/7	6/6	6/6	6/6	...	...	5·8	2·44
III.	Untreated control ...	...	...	...	...	6/6	6/6	4/6	8·24	...

Comments.—The loss in potency following centrifugation is very considerable, the second supernatant having very little residual potency.

It was considered probable that the use of the supernatant fluid made from more concentrated vaccine instead of the 5 per cent Semple's (whole brain) vaccine would have certain advantages, viz., the dose of vaccine to be administered would be proportionately smaller; or twice the quantity of antigen could be administered without corresponding increase of the dose or of brain tissue; or the number of days, for which treatment was to be administered, could be reduced.

Experiment IV.—Potency tests, using the routine technique, were carried out on (*i*) the supernatant fluid from a 10 per cent phenolized sheep brain vaccine, (*ii*) the 10 per cent vaccine, and (*iii*) a 5 per cent phenolized sheep brain vaccine made by diluting the 10 per cent vaccine. The 10 per cent supernatant and whole brain vaccines were given to the guinea-pigs in 0·5 c.c. doses on 6 alternate days and the 5 per cent whole brain vaccine in 0·5 c.c. doses daily for 12 days; other details of the technique were the same as described earlier. Results to-date have shown no significant difference in the potencies of the three vaccines.

Experiment V.—The results of the next experiment on supernatant vaccine are of interest. In this experiment treatment of guinea-pigs was begun an hour after injecting 0·5 c.c. of a 1/500 dilution of street virus (guinea-pig brain, 5th passage) into the gastrocnemius muscle. The

1st group was given 1 c.c. of the 10 per cent supernatant vaccine daily for 3 days, intraperitoneally; the 2nd group was given 1 c.c. of 5 per cent Semple's vaccine daily for 6 days, intraperitoneally; the 3rd group was left untreated. The street virus was also titrated by injecting the same dose of higher dilutions of the street virus intramuscularly into guinea-pigs. The results to-date (40 days after infection) are shown in Table VI:—

TABLE VI.

Group	Treatment	Deaths from rabies/ total	Incubation period, days
I.	1 c.c. of 10 per cent supernatant vaccine daily for 3 days beginning 1 hour after infection.	1/8	14
II.	1 c.c. of 5 per cent Semple's vaccine daily for 6 days beginning 1 hour after infection.	2/8	12,12
III.	No treatment after infection	8/8	12,14,15,16,16,16,17,18
IV.	0·5 c.c. of 1/2,500 dilution of street virus intramusculary	4/4	15,17,18,19
V.	0·5 c.c. of 1/12,500 dilution of street virus intramuscularly	0/4	...

The guinea-pigs are still under observation.

III. INTRA-NASAL INSTALLATION OF VACCINE IN THE PREVENTION OF RABIES.

Antirabic vaccine for prophylactic use in human beings and animals has always, as far as we know, been adminstered by the subcutaneous, intramuscular or intraperitoneal route; in some, so treated, particularly those previously sensitized to nerve tissue, reactions such as 'paralytic accident' occur. An investigation was undertaken to determine if the administration of antirabic vaccine by the intra-nasal route would immunize experimental animals and afford protection against a subsequent exposure to rabies infection; and whether, by the use of this route of immunization, undesirable reactions would be avoided.

Experiment I.—The first point which had to be investigated was whether live virus instilled intransally would be absorbed through the respiratory mucosa. One c.c. of a 1 per cent emulsion of live Paris fixed virus was instilled intransally on alternate days into 12 guinea-pigs. All the 12 animals came down with rabies on the 9th day, showing that the virus was absorbed quite readily.

Experiment II.—The immunizing effect of intranasal vaccination.—A group of guinea-pigs was treated by instilling 20 per cent Semple's vaccine intranasally; a dose of 1 c.c. was administered on alternate days and a total of 6 doses was given. A similar group was immunized by giving a single injection of 1 c.c. of the same vaccine, intraperitoneally.

All the animals were bled prior to treatment and again 18 days after the last dose of vaccine. Serum neutralization tests were carried out, using mice, and showed that whereas the pre-immunization samples of serum were completely devoid of rabicidal properties, the post-immunization samples, in both groups of animals, could neutralize 1,000 guinea-pig L.D.$_{50}$'s of fixed virus (C.V.S. strain).

Experiment III.—An experiment to determine the protective action of intranasally administered vaccine against a subsequent street virus infection followed. Two groups of 13 guinea-pigs each of 500 g. to 600 g. weight were immunized. The 1st group had 1 c.c. of 20 per cent phenolized vaccine instilled intranasally on 6 alternate days. The 2nd group had a single dose of 2 c.c. of the vaccine instilled intranasally on one day. The two groups were challenged with rabies street virus (guinea-pig brain : 5th passage) intramuscularly 30 days after the 1st dose of the vaccine. A 3rd untreated group, similarly infected, was included as a control. The results of this experiment are shown in Table VII:—

Table VII.

Group	Treatment	Results:
		Deaths/total
I.	1 c.c. of 20 per cent vaccine intranasally on alternate days for 6 days beginning 30 days before S.V. infection.	0/12
II.	A single dose of 2 c.c. of 20 per cent vaccine intranasally, 30 days before S.V. infection.	11/13
III.	Untreated controls.	12/12

Comments.—It would be seen that immunity of a high degree was conferred to guinea-pigs by the intranasal administration of antirabic vaccine.

Experiment IV.—Attention was also devoted to another aspect, namely, the rate at which immunity developed following intranasal vaccination. In the following experiment, groups of 10 guinea-pigs each were treated with 6 doses of 1 c.c. each of 10 per cent Semple type vaccine, given daily by the intranasal route, 30, 20 and 10 days before being injected with rabies street virus, given intramuscularly. Another group was treated with the same vaccine intranasally beginning one hour after the street virus injection and a fifth group was treated with the same vaccine intraperitoneally, beginning one hour after infection. The sixth group was not given any treatment before or after infection and served as a 'virus control'. The results obtained are given in Table VIII.

TABLE VIII.

Group	Treatment	Deaths from rabies/ total	Incubation period, days
I.	Intranasal vaccination 30 days before infection	3/9	12, 14, 49
II.	Intranasal vaccination 20 days before infection	1/9	12
III.	Intranasal vaccination 10 days before infection	5/10	9, 9, 35, 36, 38
IV.	Intranasal vaccination 1 hour after infection	10/10	9, 9, 12, 13, 14, 14,14 16, 16, 17
V.	Intraperitoneal vaccination 1 hour after inefection	5/10	9, 12, 12, 13, 16
VI.	Untreated controls	10/10	9, 9, 11, 11, 11, 11, 11, 11, 11, 12, 13

Comments.—Immunity, capable of saving at least 50 per cent of the animals against a subsequent street virus infection, which killed 100 per cent of the controls, was induced when intranasal vaccination was begun up to 10 days before infection. The prolonged incubation period in 3 out of 5 animals, dying in the 3rd group, indicated that at least 8 out of 10 animals in the group were afforded some protection.

Investigations on the problem are being continued.

IV. TREATMENT OF RABIES : LOCAL AND GENERAL.

In our previous communication we dealt with the local treatment of wounds, infected with rabies street virus, carried out 30 minutes and 4 hours after the wounds were inflicted. It was shown that cauterization with carbolic acid or nitirc acid up to 4 hours after infection had considerable saving effect in guinea-pigs. When this local treatment was, however, followed by antirabic vaccination, there was a further significant lowering in mortality.

As the subject is a very important one in regard to the prophylaxis of rabies, we have extended our investigations on this subject and the following protocols, among other observations, record the results of further experiments on the efficiency of cauterization as a first-aid treatment.

For this purpose, firstly, we have studied the effect of the application of carbolic acid and nitirc acid (*i*) alone, and (*ii*) followed by antirabic vaccination, 4 hours and 24 hours after infection; and secondly, the problem has been studied in regard to the use of hyperimmune antirabic serum as a local and general treatment, with and without the use of antirabic vaccine. Working on these lines it has been possible to get a fairly good idea of the value of various forms of treatment used in the prophylaxis of rabies.

Experiment I.—Encouraging reports have recently appeared on the use of detergents (Shaughnessy and Zichis, 1954) as an effective first-aid treatment for bites inflicted by rabid animals. A detergent 'Cetavlon' (cetyltrimethylammonium bromide) kindly supplied by I.C.I. (India) Ltd., was tested along with the other agents in this experiment.

Sixty guinea-pigs were infected with a locally isolated strain of street virus and treated 4 hours later as shown in Table IX:—

TABLE IX.

Protective effect of corrosive and non-corrosive agents used in the local treatment of rabies infected wounds.

Treatment	Deaths/ total	Percentage mortality	Survival period
Carbolic acid	4/11	36	12, 12, 14, 18
Nitric acid	3/10	30	16, 19, 20
Methylated spirit	8/10	80	11, 12, 12, 12, 13, 14, 15, 13
'Cetavlon' 1 per cent solution	8/10	80	11, 11, 12, 12, 17, 17, 19, 22
Controls	11/12	92	11, 12, 12, 13, 13, 15, 16, 18, 19, 20, 22

Difference in denominators is due to deaths from other causes.

Comments.—Cauterization of wounds with carbolic acid or nitric acid, 4 hours after infection, is of considerable value in preventing the onset of rabies. Application of 1 per cent solution of 'Cetavlon' or of methylated spirit under the same conditions is much less effective.

Experiment II. Repetition of experiment I.—In this experiment the time interval between infection and local treatment was increased to 24 hours Forty-five guinea-pigs of 500 g. weight were infected with a locally isolated strain of street virus, recovered from a dog, and treated as shown in Table X:—

TABLE X.

Protective effect of cauterization 24 *hours after infection with street virus.*

Treatment	Deaths/ total	Percentage mortality	Survival period
Carbolic acid	10/14	71	12 13 13 13 14 14 15 15 18 41.
Nitric acid	12/15	80	11 12 13 13, 14, 14, 14, 15, 15, 15, 16, 17.
Controls	14/15	93	10, 10, 10, 11, 11, 12, 12, 13, 13, 14, 15, 15, 20, 28.

Comments.—Cauterization of wounds with carbolic acid or nitric acid 24 hours after infection is of limited value.

In the previous experiments the value of cauterization alone was investigated. In the protocols presented below the effect of cauterization followed by antirabic vaccination is recorded.

Experiment III.—One hundred and twenty guinea-pigs of 500 g. to 600 g. weight were infected with street virus and divided into groups of 15 animals and treatment was carried out as shown in Table XI:—

TABLE XI.

Protective effect of (i) cauterization, (ii) cauterization followed by antirabic vaccine, and (iii) vaccine alone, 24 *hours after infection with street virus:*

Treatment	TREATMENT STARTED 24 HOURS AFTER INFECTION:	
	Deaths/total	Percentage mortality
(*i*) Nitric acid	13/15	87
Carbolic acid	11/15	73
Methylated spirit	14/15	93
(*ii*) Nitric acid *plus* vaccine	6/15	40
Carbolic acid *plus* vaccine	6/15	40
Methylated spirit *plus* vaccine	11/15	73
(*iii*) 5 per cent Semple's vaccine	9/15	60
Controls	15/15	100

Comments.—Cauterization of wounds with carbolic acid or nitric acid, 24 hours after infection with street virus followed by antirabic vaccine, significantly diminished the chances of developing rabies, the mortality being reduced from 100 per cent in untreated controls to 40 in animals treated with carbolic acid or nitric acid *plus* vaccine. With vaccine alone 60 per cent of the animals died.

Comparatively poor results obtained with cauterization alone, in this experiment are probably due to the excessive dose of a highly virulent strain of street virus, causing 100 per cent mortality in untreated animals used as test infection.

Experiment IV.—This was designed to assess the saving effect of hyperimmune antirabic serum administered by (*i*) topical application *plus* infiltration into the site of wound, and (*ii*) by intraperitoneal injection with or without antirabic vaccine treatment, 4 hours and 24 hours after infection. One hundred and twenty-three guinea-pigs were infected with street virus and treated as shown in Table XIII. In the case of animals treated with immune serum followed by antirabic vaccination, vaccine treatment was commenced 24 hours after the injection of serum.

The serum used was prepared at the Central Research Institute, Kasauli, and had a titre of approximately 1 in one million, i.e., one c.c. of serum neutralized 1 c.c. of rabid virus (C.V.S. strain) containing one million LD_{50} doses for a guinea-pig of about 300 g. weight. The result of the potency test of the serum, using guinea-pigs as test animals, is given in Table XII:—

TABLE XII.

Dilution of virus	Dilution of serum	Survivals/total	LD_{50}
1 in 5,000	1 in 100	4/4	$10^{-3\cdot5}$
1 in 5,000	1 in 1000	4/4	
1 in 5,000	1 in 10,000	0/4	
Virus titration			
10^{-5}	10^{-6}	10^{-7}	10^{-8}
4/4	3/3	0/4	0/4

Number of LD_{50} used in the test ...316.
1 in 3,162 dilution of serum neutralized...316 LD_{50}.
Potency of serum...998,600 LD_{50} guinea-pig doses.

Conclusion.—Evaluation of various forms of antirabies treatment in experimental rabies shows that cauterization of rabies-infected wounds with carbolic acid or nitric acid within 4 hours of infection has a marked saving effect. The same treatment carried out 24 hours after infection is much less effective.

Application of methylated spirit or of 1 per cent solution of detergent 'Cetavlon' does not have any notable effect on reducing the chance of developing rabies.

Topical application of hyper-immune antirabic serum combined with infiltration of the serum into the site of the wounds, 4 hours after infection, has little or no saving action. Intraperitoneal injection of serum is of some value but the results obtained with serum treatment alone, apart from prolonging the period to death, are poor as compared with vaccine therapy.

Cauterization of wounds with carbolic acid or nitric acid followed by a course of anitrabic vaccine is of considerable value in preventing rabies, the mortality being reduced from 100 per cent in untreated animals to 40 per cent in the groups treated with carbolic acid or nitric acid *plus* vaccine.

Rabies can be prevented in experimental animals by antirabic vaccine given within 24 hours of injection of virus, but somewhat better results are achieved by giving hyper-immune antirabic serum, 24 hours after infection, followed 24 hours later by a course of antirabic vaccine, the mortality ratio in untreated, vaccine-treated and vaccine-cum-serum treated animals being of the order of 93:41:30, respectively. The use of potent rabicidal sera in combination with antirabies vaccination would appear to be a promising line of treatment. If these results of serum thereapy in experimental rabies are confirmed in field trials in human subjects, the ideal antirabies treatment would be (*i*) cauterization of bites with carbolic acid or nitric acid as a 'first aid' treatment (this itself may have a great saving effect if carried out not later than 24 hours after bite), and (*ii*) injection of concentrated and

TABLE XIII.

Protective effect of hyper-immune antirabic serum.

Treatment	TREATMENT STARTED 4 HOURS AFTER INFECTION:			TREATMENT STARTED 24 HOURS AFTER INFECTION:		
	Deaths/ total	Percentage mortality	Survival period.	Deaths/ total	Percentage mortality	Survival period.
1. Serum applied locally 0·5 c.c. *plus* serum infiltrated into the site of wound 1·5 c.c.	11/14	79	18,20,20,20,21,21 21,21,21,21,21.	10/12	83	11,14,17,19,20,20 22,24,26,30.
2. Serum applied locally 0·5 c.c.; serum infiltrated into the site of wound 1·5 c.c.; serum given intraperitoneally 1·5 c.c.	8/15	53	17,19,20,21,21,22 22,22.	7/11	64	18,19,20,22,24,24 22.
3. Semple's vaccine 1·5 c.c. daily, intraperitoneally for 6 days.	5/15	33	11,14,14,17,17	5/12	41	12,12,15,15,16.
4. Serum applied locally 0·5 c.c. serum infiltrated into site 1·5 c.c.; Serum given intraperitoneally 1·5 c.c. vaccine 6×1·5 c.c. intraperitoneally.	7/14	50	14,18,19,19,20,26 45.	3/10	30	16,18,19.
5. Controls.	14/15	93		14/15	93	11,11,11,13,13,13 14,14,15,15,16 16,17,17.

Comments: Topical application of hyper-immune antirabic serum combined with infiltration into the site of the wound is of very limited saving value, if any. Intraperitoneal injection of hyper-immune serum within 24 hours of infection is of value in the treatment of rabies-infected animals but the results obtained are poor as compared with vaccine therapy alone.

refined rabicidal serum within 24 hours of the bite, followed 24 hours later by antirabies vaccination.

V. CONCENTRATION AND PURIFICATION OF THE ANTIRABIES SERUM.

Further work, has been carried out on the concentration and purification of antirabic serum by dialysis of the inorganic constituents of the plasma, iso-electric precipitation and final precipitation with ammonium sulphate. The results show that by this method a concentration of four to six times and a recovery of 80 to 90 per cent of antiviral bodies is obtained. As far as purification is concerned, half the amount of total nitrogen is removed. The total solids vary from 15 to 18 per cent.

Concentration and purification by the method usually used for the concentration of diphtheria and tetanus antitoxins has given much lower recovery of the antiviral bodies (30-40 per cent) than the method described above.

3. Polio Research Unit under Dr. P. V. Gharpure at the Grant Medical College, Bombay.

The report is presented under the following heads to maintain the form of all the former annual reports:—

A. EPIDEMIOLOGICAL STUDIES

B. VIRUS STUDIES.

Work was carried on along the directions of the Virus Diseases Advisory Committee and the Scientific Advisory Board.

A. EPIDEMIOLOGICAL STUDIES.

(1) Clinical work in Bombay and suburbs and

(2) Field work.

1. *Clinical work:*

One hundred and fifty six cases were studied from 1-9-1954 to 30-9-1955 in Greater Bombay

TABLE I.

Hospital-wise distribution of cases.

Name of the hospital	Number of cases
Byramjee Jeejeebhoy Hospital for Children	39
Jerbai Wadia Hospital for Children	35
Arthur Road Hospital	77
Municipal General Hospital Sion	3
Breach Candy Hospital	1
Bombay Hospital	1
Total	156

Fourteen cases were declared as non-polio cases after due investigation.

Tables II to V show the distribution of cases geographically, age and sex-wise and month-wise.

TABLE II.

City ward	Number of cases	Suburbs	Number of cases
A	6	Khar	1
B	6	Santacruz	5
C	11	Villeparle	4
D	8	Andheri	3
E	30	Varsova	2
F	17	Jogeswari	2
G	23	Vadala	1
		Kurla	1
		Bhandup	2
		Ghatkopar	1
Total	101		22

TABLE III.

Imported cases.

Place	Number of cases	Place	Number of cases
		Dolkhan	1
		Gorai	1
		Gowari	1
Malad	2	Kalsule	1
Kandivli	1	Kalva	1
Bassein	1	Khillarwadi	1
Bulsar	2	Kinale	1
Mulund	1	Koregaon	1
Thana	4	Mangaon	1
Kalyan	2	Mankhurd	3
Ambernath	2	Morolgaon	1
		Panvel	1
		Rahimatpur	1
		Rayam	1
		Sapala	1
		Subedar	1
		Total	33

Six specimens of stools were received by post from Baroda.

TABLE IV.

Month-wise distribution of cases.

1954		1955	
Month	Number of cases	Month	Number of cases
		January	8
		February	1
		March	5
September	28	April	8
October	13	May	15
November	10	June	16
December	3	July	23
		August	15
		September	11
Total			156

TABLE V.

Age and sex-wise distribution.

Age	Males	Females	Total
0 to 6 months	5	3	8
6 - 12 ,,	16	15	31
13 - 24 ,,	32	27	59
25 - 36 ,,	14	8	22
37 - 48 ,,	7	8	15
49 - 60 ,,	2	1	3
6 years to 9 years	6	2	8
10 ,, 14 ,,	2	1	3
15 ,, 19 ,,	2	0	2
20 ,, and above	3	2	5
Total	89 (57 per cent)	67 (43 per cent)	156

Fatalities.—There were 16 fatal cases. Fatal cases were predominantly bulbar cases.

Field work: Home visits.—Two hundred and seventy home visits were made. The visits were made to study the environmental conditions of patients and for collection of materials for virus studies.

Materials collected.—This year the collection of material was restricted to stools and sera only. One hundred and fortyfour samples of fæces from patients and 65 fæces from contacts were collected. Details regarding sera are given under serological studies.

Serological studies.—To obtain the spectrum of the antibody levels among the population in this country, steps have been taken to collect sera from different age groups. 59 sera were collected at random and were forwarded to Dr. David Bodian, U.S.A., for the study of antibody contents, the results of which are being awaited.

Sixteen samples of sera were collected from patients having paralytic poliomyelitis and their contacts.

Seventy nine sera collected in September in Delhi have been received from Dr. (Mrs.) Bhatia of the Lady Hardinge Medical College, New Delhi.

Study of minor illnesses-during the epidemic and interoepidemic periods.—This study included collection of data regarding the incidence of minor illnesses in children below the age of 6 years in the population of certain localities of Bombay. This was undertaken with a view to find out the relation between the incidence of cases of paralytic poliomylitis and the incidence of minor illnesses during the particular period of the year. These data will be analysed after one year.

The number of cases of poliomyelitis in the period of this report compared with those of the previous years studied by this unit will be seen from the figures given below:—

Year	Number of cases	Year	Number of cases
1949	253	1953	92
1950	133	1954	259
1951	75	1955 (up to 30-9-55)	102
1952	169		

B. VIRUS STUDIES.

The report is presented under the following heads:

- (*i*) Fresh isolations
- (*ii*) Passage
- (*iii*) Serology
  - (*a*) Preparation of type-specific sera, and
  - (*b*) Typing of virus strains.

(*i*) *Isolation of fresh strains of polio virus.*—Table VI summarizes the materials collected and the isolations made from them. Twenty-eight new strains of polio virus were isolated.

(*ii*) *Passage work for maintenance of strains isolated in previous years.*—It has not been possible to carry out passage in monkeys of all the strains isolated so far. This was partly due to greater attention being directed towards tissue culture work. It has been possible to establish all the strains in possession of the unit in tissue culture. The third passage infected tissue culture fluids have been stored at-20°C. for further study.

TABLE VI.

	BOMBAY AND SUBURBS:						Outside Bombay	Other than hospital	Totals
	BJ*	JW	ARH	BRH	SH	BH			
Collection	77	38	126	1	3	1	21	10	277
Tested for polio virus in monkeys ...	48	7	43	0	0	0	12	6	116
Tested for polio virus in tissue culture	17	8	17	1	3	1	2	1	50
Positive isolations	10	2	7	0	0	0	6	3	28
Awaiting results in monkeys ...	11	0	12	0	0	0	0	0	23
Awaiting results in tissue cultures ...	10	2	4	0	2	1	2	1	22
Awaiting inoculations ...	12	23	66	0	0	0	7	3	111

*BJ = Byramjee Jeejeebhoy Children Hospital.
JW = Jerbai Wadia Children Hospital.
ARH = Arthur Road Hospital (City Fever Hospital).

BRH = Breach Candy Hospital.
SH = Municipal General Hospital, Sion.
BH = Bombay Hospital.

(*iii*) (*a*) *Preparation of type-specific sera.*—Following the method detailed in last year's annual report, efforts were made to prepare antisera against all the 3 types of poilo virus. It has been possible to prepare antisera against type I and type II and that against type III is under way. Table VII summarizes the results of studies carried out in tissue culture of the 50 per cent serum dilution protective end-point of the antisera prepared.

(*iii*) (*b*) *Typing of virus strains.*—Typing of the strains of polio virus isolated in this unit was carried out in tissue cultures. Tissue culture methods have now been developed in this unit and most of the work was carried out by employing monkey-kidney tissue. Monkey-testicular tissues have also been employed to grow polio virus. Monolayer monkey kidney cell cultures were prepared following a modification of Younger's technique for preparation of trypsin dispersed cell suspension. Under nembutal anæsthesia *rhesus* monkeys were exsanguinated and kidneys removed aseptically. The connective tissues and pelvic fat removed as far as possible, the kidneys were minced with uterine scissors into small pieces (4 mm. to 5 mm. in diameter) and the minced tissue washed well with Hank's balanced salt solution to remove the adhering r.b.c. The washed tissue was suspended in 25 c.c. of trypsin solution per kidney and incubated at 37° C for 30 minutes in the trypsinization flask. The supernate after incubation was collected in a flask kept in ice-bath and fresh trypsin was added to the tissues and agitated for 10 minutes by placing the flask over a Mag Mix. The supernate was collected and added to the same flask kept in the ice-bath. Addition of fresh trypsin to the tissues, agitating the tissues for 10 minutes over the Mag Mix and decanting the supernate into the flask were carried out for at least 8 to 10 times, by which time most of the parenchymal cells of the kidney have separated out from the tissues. The collected fluid containing the cells was then centrifuged at 200 r.p.m. for 30 minutes, and the sediment suspended in a nutrient medium consisting of Hank's balance solution, normal inactivated monkey serum, 0·5 per cent lactalbumin enzymatic hydrolysate with antibiotics (penicillin and streptomycin), so as to have 0·8 million cells per millilitre. This suspension is kept overnight in ice-bath at 2°C. to 4°C. for stabilization. Next day the cell suspension is centrifuged at 200 r.p.m. for 30 minutes, and the sediment re-suspended in the nutrient medium so as to contain 0·8 million cells per c.c. 0·5 c.c.. of this diluted cell suspension was pipetted out into test tubes (5″ × 5/8″) with air-tight rubber corks. These are incubated at 37°C. for 7 to 9 days at a slight inclination to the horizontal. In this period, a zone of confluent kidney epithelium covers the area of the tube in contact with the cell suspension and the pH of the medium drops to 6·8. The medium is changed and fresh medium added when the tubes are ready for virus isolation, assay and other studies.

All the strains of virus isolated in this unit were successfully passed to three passages in kidney tissue cultures and all showed the typical cytopathogenic changes. Fluids from the third passage were tested in tissue culture against each of the three type-specific poliomyelitis antisera. 0·2 c.c. of 10^{-3} dilution of tissue culture fluid was mixed with 0·2 c.c. of each antisera diluted previously to 1:10 or 1:5 (depending upon the titre of the serum) with Hank's balanced salt solution. After incubation for one hour at room temperature, 0·2 c.c. of each serum-virus mixture was inoculated into two tissue culture tubes. The cultures were incubated in a stationary position and observed daily for six days. The virus was

TABLE VII.

Fifty per cent dilution serum protection end-point of antisera prepared in monkeys.

Serum	Tissue culture infection dose of virus used	FINAL DILUTION IN SERUM										Fifty per cent serum dilution end-point
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	
Type I ...	100	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	1/2	2/2	1:512
Type II ...	100	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	1/2	2/2	1:512

identified by the serum which neutralized its cytopathogenic property. Table VIII records the types of polio virus isolated in this unit during the period 1950 to 1954.

TABLE VIII.

Types of polio virus isolated during the period 1950-54.

Type	YEAR:					Total
	1950	1951	1952	1953	1954*	
I	1	1	2	7	34	45
II	...	1	...	1	...	2
III	...	...	...	...	1	1
Totals	1	2	2	8	35	48

4. Inquiry on the incidence, nature and types of coxsackie virus infections in Bombay City under Dr. N. M. Purandare at the Seth G. S. Medical College, Bombay.

Attempts have been made to isolate strains of Coxsackie viruses from samples of fæces, blood, nerve tissue, etc., from patients suffering from various febrile maladies with suspected virus etiology and from common house flies (*Musca sp.*) and fruit flies (*Drsophylla sp.*), collected from different localities in Bombay. So far 32 strains of coxsackie viruses have been isolated by inoculation of the collected material into newborn albino mice. Out of these, 22 strains of Coxsackie viruses were studied further for their antigenic pattern. During this year attempts were made to cultivate some of these strains in tissue culture.

(1) *Collection of material.*—A collection of material from 82 cases was made from 1st October 1954 to-date, thus making a total of 248 cases from the beginning of the work. All the collected material was kept frozen in a freezing cabinet at—20° C. to await tests. The distribution of the material from these 82 cases was as follows:—

(*a*) Fæcal samples from 36 cases admitted at either Bai Jerbai Wadia Hospital for Children or at K. E. M. Hospital, Parel. The patients were clinically diagnosed as suffering from paralytic poliomyelitis encephalitis, etc.

(*b*) Fæcal samples from nine cases admitted to Irwin Hospital, New Delhi, and one fæcal sample from a suspected case of poliomyelitis at Jamshedpur. These samples were received from Dr. J. A. Kerr, Director, Virus Research Centre, Poona. One fæcal sample from a case of suspected Coxsackie virus infection was sent by the Secretary, Indian Council of Medical Research, New Delhi.

(*c*) Brain and/or spinal cord material from *15 cases* of encephalitis, myelitis, etc., autopsied at K. E. M. Hospital and *two cerebrospinal fluid* samples from two cases of suspected poliomyelitis at K. E. M. Hospital, Parel.

(*d*) Total number of approximately about 300 flies, which were collected at ten different times at Kurla with the help of Dr. P. J. Deoras, Assistant Director, Haffkine Institute, Parel. About 250 fruit flies were collected at ten different localities in Bombay.

(2) *Isolation of strains of Coxsackie viruses.*—Thirty-six out of 47 fæcal samples, suspensions from about 300 house flies and about 200 fruit flies and one sample of serum collected from a suspected case of encephalitis in acute phase, were inoculated subcutaneously into litters of newborn albino mice. Out of these samples ten different strains of Coxasckie viruses were recovered bringing the total of strains isolated to-date to 32. In six of these ten cases, the virus was isolated from fæces of six cases, clinically diagnosed as suffering from poliomyelitis and admitted at Bai Jerbai Wadia Hospital for Children. Two strains were isolated from adults admitted at K. E. M. Hospital and were suffering from pleurodynia and quadriplegia, respectively. One of the remaining two strains was isolated from a stool sample of a patient suffering from ' Mystery Disease ' at Irwin Hospital, New Delhi, while the other was from a child admitted at M. G. Hospital, Sion, and clinically diagnosed as suffering from suspected encephalitis.

(3) With a view to isolating any other mouse adaptable virus in addition to Coxsackie virus the following material (collected in the previous and this year) consisting of suspensions of 26 brain and spinal cord samples, two samples of C. S. F. from the suspected cases of poliomyelitis and suspensions of about 500 flies were inoculated into groups of six adult mice (five to six weeks old) by intracerebral route. No virus was recovered from these samples.

(4) *Preparation of high titre immune sera for neutralization tests.*—Immune sera against four out of ten recently isolated strains of Coxsackie viruses were prepared in adult albino mice of about ten week old, following the methods advocated by Melnick (personal communication). Accordingly, four batches of twenty mice each were inoculated, respectively, with mouse muscle suspensions of these strains by intraperitoneal route. After the requisite number of injections the mice were bled from the heart, the sera were separated and were frozen at—20° C. Titre of neutralizing antibody in the samples of antisera was then determined.

(5) *Cross neutralization tests.*—In order to find out the antigenic relationship among the locally isolated strains, cross-neutralization tests were carried out on four of the recently isolated strains of Coxsackie viruses following the methods described by Melnick and Ledinko (*Jour. Exper. Med.*, **95,** pp. 463-482, 1950). These cross-neutralization tests with the hyperimmune sera prepared against known types in this laboratory revealed that three out of four strains could be typed into types 4, 10 and 12, respectively, of Dalldorf's group A. The remaining strain could not be typed with the six known type antisera against strains of Coxsackie viruses. In addition to these four recently isolated strains, six remaining strains isolated previously were subjected to a similar study. Out of these six strains four strains could be classified as two belonging to the type 1 and 2 to the type 10, respectively. The remaining two strains were untypable. Thus, a study of antigenic pattern of 22 out of 32 strains isolated so far revealed the following incidence of types (Table I).

TABLE I.

Antigenic pattern of locally isolated strains of Coxsackie viruses.

	Dalldorf's group A							Group B
Types	1	3	4	10	12	14	Unidentified	
Number of strains	3	1	8	4	2	1	3	*Nil*

(6) *Cultivation in tissue culture tubes.*—In order to find out the presence or otherwise of poliomyelitis virus in the fæcal suspensions, collected for the isolation of Coxsackie virus, 66 fæcal samples were inoculated in tissue culture tubes containing *rhesus* monkey kidney cells. This was carried out in collaboration with the work on monkey kidney cell suspensions in this Department. Out of 66 samples 16 showed a cytopathogenic agent indicating thereby presence of a virus. Nine of these 16 strains were of poliomyelitis virus type 1 (isolated and typed by Dr. P. N. Bhatt from Poliomyelitis Unit at Grant Medical College, Bombay), two strains could not be identified and the remaining five strains are in the process of identification. One of these two unidentified strains was isolated from a case of ' Mystery Disease ' from New Delhi. No virus could be recovered from this specimen by intracerebral inoculation into monkeys or adult and newborn albino mice. The strain is sent to Dr. J. L. Melnick, Yale University, School of Medicine, for identification.

Attempts made to grow some of these local strains of Coxsackie viruses in monkey kidney cell suspensions have so far yielded negative results. As a control to these experiments a strain of Coxsackie viruses (Grigg strain) adapted to the monkey kidney cells, which was kindly sent to us by Dr. J. L. Melnick, was inoculated in the monkey kidney cell suspension tubes. This strain was found to grow well in the tubes showing a clear-cut cytopathogenic effect on the epithelial cells. Three attempts were made to try to grow trypsinized mouse kidney cells in tubes with Hank's solution, with negative results.

5. Rabies inquiry under the Director, Pasteur Institute, Coonoor.

1. *A comparative study of the value of five per cent Semple vaccine prepared in distilled water and in normal saline in human treatment.*—It has been reported by D'Silva and Ahuja (1951, 1954) that when distilled water is substituted for normal saline in the preparation of five per cent Semple vaccine the antigenicity of the vaccine is considerably increased. The Virus Diseases Advisory Committee of the Indian Council of Medical Research at its meeting held on the 24th November, 1953 passed the following resolution:

' The Committee considered the report on the observations made by the Director, Central Research Institute, Kasauli, on the use of distilled water as the menstruum in the preparation of antirabic vaccine in place of normal saline solution. They noted with interest that the use of the vaccine in the field during the year 1951-52 had resulted in reduction in mortality from hydrophobia. The Committee considers it of importance that other institutes engaged in the preparation of antirabic vaccine should also carry out similar investigations to assess the value of five per cent Semple's vaccine prepared in distilled water'.

Based on this recommendation the Institue supplied five per cent Semple vaccine prepared in distilled water (DWV) from 1st March, 1954 to 31st October, 1954. Among the 12,974 patients treated with this vaccine and whose case cards were returned, three developed post-treatment complications giving an incidence rate of one in 4,325. The Institute, which has a very good follow-up system, has never had more than one case of neuroparalytic accident during any year. In some years there have been no cases at all. Among the 2,96,569 patients who received a complete or incomplete course of treatment with vaccine prepared in normal saline during the years 1933 to the end of 1953 and for whom complete case records are available, 13 patients developed post-treatment complications giving a rate of incidence of 1 in 22,813. In view of the comparatively high incidence of accidents following treatment with the vaccine prepared in distilled water its use was discontinued from 1st November, 1954.

With a view to assess the value of DWV in human treatment under controlled conditions the mortality among those treated with this vaccine and those who refused treatment has been investigated. These include persons bitten by infective, proved or presumably rabid animals. These findings are summarized in Table I together with similar data obtained among persons treated with vaccine prepared in normal saline (SV) for comparison.

These findings do not indicate any superiority of the vaccine prepared in distilled water over that prepared in normal saline.

2. *A comparative study of the antigenic value of five per cent Semple vaccine prepared in distilled water and in normal saline in animals.*—D'Silva and Ahuja reported that antirabic vaccine prepared in normal saline as the menstruum was as effective in affording protection in the pre-infection treatment of street virus infected guinea-pigs as the vaccine prepared in distilled water. They, however, claimed that the latter vaccine immunized more quickly and gave better protection in the post-infection treatment of animals. Experiments were carried out to confirm the work under standard conditions.

0·1 c.c. of a 1:80 dilution of the lyophilized canine submaxillary gland suspension of the NYC strain and 0·1 c.c. of a 1:320 dilution of the canine submaxillary gland suspension of the marimuthu strain were tried. The latter strain was isolated from the saliva of a patient (marimuthu) who died of hydrophobia at the Institute.

The DWV and SV used in these experiments were prepared from the same brain. A fixed virus infected sheep brain was divided longitudinally into two equal parts. One half was used for the preparation of DWV and the other for SV.

The results are summarized in Tables II and III.

TABLE I.

	BITTEN BY INFECTIVE ANIMALS:				BITTEN BY INFECTIVE, PROVED RABID AND PRESUAMBLY RABID ANIMALS:			
	SV	Per cent	DWV	Per cent	SV	Per cent	DWV	Per cent
Number of persons observed ...	331		57		4,191		612	
Number completely treated ...	213		31		3,753		533	
Deaths among completely treated ...	16	7·5	2	6·5	16	0·42	2	0·37
Number incompletely treated ...	20		5		99		30	
Deaths among incompletely treated	5	25	1	20	5	5·05	1	3·33
Number who died during treatment	13		2		13		2	
Number untreated	85		19		326		47	
Deaths among untreated ...	36	42·3	8	42·1	36	11·04	8	17·0
Number untreated and those who died during treatment	98		21		339		49	
Deaths among untreated and those who died during treatment ...	49	50	10	47·6	49	14·45	10	20·4

*Two deaths after complete treatment with DWV are not included as exact details regarding other persons and animals bitten by the same dogs were not available.

TABLE II.

Group	Challenge	Nature of treatment	Mortality due to rabies	Average incubation period, days
I	NYC 1:80	DWV, 0·3 c.c. daily, s/c, for 7 days before challenge ...	0/15	...
II		SV, 0·3 c.c. daily, s/c, for 7 days before challenge	0/15	...
III		DWV, 1·0 c.c. daily, s/c, for 7 days, treatment started 1 hour after challenge	9/14	13·2
IV		SV, 1·0 c.c. daily, s/c, for 7 days treatment started 1 hour after challenge	11/15	14·7
V		Untreated control	14/15	23·5

Number of intramuscular LD_{50} given 11·3.

TABLE III.

Group	Challenge	Nature of treatment	Mortality due to rabies	Average incubation period, days
I	Marimuthu 1:320	SV, 0·3 c.c. daily, s/c, for 7 days before challenge	4/12	38
II		DWV, 1·0 c.c. daily, s/c, for 7 days, treatment started 1 hour after challenge	11/12	15
III		SV, 1·0 c.c. daily, s/c, for 7 days, treatment started 1 hour after challenge	11/12	14·9
IV		Untreated control	11/12	19·4

Number of intramuscular LD_{50} given as challenge 12·6.

Under the conditions of the experiments reported, it has not been possible to demonstrate that vaccine prepared in distilled water immunizes more quickly and gives better protection in the post-infection treatment of animals compared with the vaccine prepared in saline.

3. *Studies on antirabies serum.*—Koprowski *et al.* in a study which is quantitative and parallels closely the events occurring in nature reported that with one injection of antiserum it was possible to protect a majority of the animals challenged with 20 to 40 LD_{50} of canine salivary gland infected with street virus in contrast to treatment with Semple type of vaccine which gave no evidence of protection. They also found that a combined antiserum *plus* vaccine treatment of exposed animals seemed neither to enhance nor to decrease the protective power of antiserum.

The value of serum in treatment was investigated using Lederle's antirabies serum. The virus used for challenge was the lyophilized canine submaxillary gland suspsension of the NYC strain street virus used by Koprowski in his study as well as the Marimuthu strain. The results are summarized in Tables IV, V and VI:—

TABLE IV.

Group	Challenge	Nature of treatment	Mortality due to rabies	Average incubation period, days
I	NYC 1:40	Serum, 0·25 c.c., i/m, 1 hour after challenge	23/25	26·9
II		Untreated control	24/25	18·8

Number of intramuscular LD_{50} given as challenge 16.

TABLE V.

Group	Challenge	Nature of treatment	Mortality due to rabies	Average incubation period, days
I	NYC 1:80	Serum, 0·25 c.c., i/m, 1 hour after challenge ...	15/15	34·4
II		Serum, 0·25 c.c., s/c, 24 hours after challenge ...	15/15	26·4
III		Serum, 0·25 c.c., infiltrated locally, 1 hour after challenge	14/15	28·5
IV		Untreated control ...	13/14	17·1

Number of intramuscular LD_{50} given as challenge: 8

TABLE VI.

Group	Challenge	Nature of treatment	Mortality due to rabies	Average incubation period, days
I	Marimuthu 1:320	Serum 0·25 c.c., i/m, 1 hour after challenge	12/12	28
II		Untreated control	11/12	19.4

Number of intramuscular LD_{50} given as challenge: 12

Under conditions, which closely resemble those described by *Koprowski et al.*, it was not possible to demonstrate any protection among animals challenged with the NYC or Marimuthu strains of virus and treated with serum. Antirabies serum alone, while prolonging the incubation period, had no saving effect with the challenge doses employed.

4. *Determination of the virus content of submaxillary glands of animals which die of natural rabies infection.*—An investigation has been carried out to determine the virus content of (*i*) the submaxillary glands and (*ii*) the brains of animals which die of natural rabies infection. The purpose is to determine what proportion of animals whose brains are positive for Negri bodies show the presence of virus in the salivary glands and whether there is any correlation between the occurrence of Negri bodies, the virus content of the brain and the salivary glands.

The virus content of the submaxillary glands of 92 dogs, one jackal, two cats, one cow, three calves and one goat, which were suspected to have died of rabies, has been titrated in mice. The results are summarized in Table VII.

TABLE VII.

Group								Per cent
(*i*)	Brain:	Negri	+ ;	brain:	Virus	+	...	58
(*ii*)	,,	,,	— ;	,,	,,	±	...	7
(*iii*)	,,	,,	— ;	,,	,,	—	...	34
(*iv*)	,,	,,	+ ;	Gland:	,,	+	...	34
(*v*)	,,	,,	+ ;	,,	,,	—	...	25
(*vi*)	,,	,,	— ;	,,	,,	+	...	5
(*vii*)	,,	Virus	+ ;	,,	,,	+	...	59
(*viii*)	,,	,,	— ;	,,	,,	—	...	30

The infective titre of the submaxillary glands varied from 10^{-1} to $10^{-5.5}$.

The fate of the treated and untreated persons bitten by animals showing the presence of virus in the submaxillary glands is being followed up and should give valuable information regarding the value of vaccine therapy.

5. *Bat rabies.*—It has been reported that the first case of hydrophobia following an insectivorous bat bite occurred in Srikakulam District of the Andhra State.

With the co-operation of the local authorities bats were shot in the area where the patient was bitten and examined for the occurrence of natural rabies infection among the bat population. So far, 12 bats have been examined. Negri bodies and the virus were not demonstrable in the brains of the bats.

6. Study of the lymphogranuloma psittacosis group of viruses with special reference to 'Ornithosis' under Dr. J. B. Shrivastav at the Medical College, Nagpur.

The local parrot population has been found to be free from infection. The local pigeon population shows antibodies against the viruses belonging to the psittacosis-ornithosis group. It was observed that the heat-stable group-specific antigen of L. G. V. virus and psittacosis virus are similar qualitatively but the quantity of the antigen varies on these two viral bodies. An attempt has been made to culture trachoma virus on human conjunctival tissues grafted on chorioallantois of developing chick embryo.

1. *Infection spectrum in the local bird population.*—Parrots and pigeons of both sexes and juvenile as well as adults were caught in and around Nagpur, were bled from the wing vein and sera subjected to a C.F. test against group specific heat stable psittacosis antigen.

Out of 14 parrots investigated none showed any compliment fixing antibodies for psittacosis group of viruses, while out of 13 pigeons investigated three showed antibodies to a titre of 1:20 and two showed antibodies to a titre of 1:40. All these pigeons, though suffering from intestinal worms, were apparently healthy. Attempts at isolation of virus from them have not been successful so far.

This survey work is being continued.

2. *Antigenic relationship of L.G.V. and psittacosis virus.*—It is well known that the viruses belonging to this group have a common group specific heat stable antigen which is responsible for cross serological reactions

between the various members of this group and their antisera. It was observed that this antigen on the L.G.V. virus behaves differently than that on psittacosis virus. L.G.V. antigen with its homologous serum gives a lower titre than psittacosis antigen with L.G.V. serum. This makes one feel that though qualitatively similar, they are different quantitatively on the two viral bodies; i.e. less on L.G.V. virus. Attempts have been made in this work to prove this by:—

(1) Adsorbing separately the L.G.V. serum with the two antigens (L.G.V. and psittacosis heat stable fractions) and titrating the residual antibody left by the standard psittacosis heat stable antigen. In a series of six experiments of this nature, it was found that on adsorption the group specific (H.S.) antigen of psittacosis virus lowered the initial titre about six times as compared with the corresponding lowering of titre achieved by the antigen on L.G.V. virus.

(2) On doing a quantitative C.F. test as suggested by Fulton and Dumbell (1949) with L.G.V. serum against these two antigens, in a series of four experiments, it was found that the psitttacosis antigen fixed complement approximately 3·5 times more than that fixed by L. G. V. antigen. On the basis of this antigenic study one feels that perhaps the two antigens, though qualitatively similar, are present in different quantities on these two viral bodies.

This work has been presented in form of a paper at the Sixth Annual Meeting of the Indian Association of Pathologists held at Nagpur.

3. *Culture of trachoma virus on human conjunctival tissues grafted on chorioallantois of developing chick embryo.*—Culture of trachoma virus by the common and standard techniques of virus culture has not been possible so far. Claims have been made by several workers, but they have not stood the test of time. Propagation of virus by animal inoculation is also a difficult and time consuming process as the virus will only grow on conjunctivæ of humans, apes, and baboons.

In this inquiry an attempt has been made to culture the virus on small pieces of human conjunctival tissues grafted on chorioallantois of 10-12 days old developing chick embryos. The technique adopted for making the grafts, in general, was the same as suggested by Sezer (1950, 1953) and Kirber (1953) for the culture of the virus of keratoconjunctivitis. Preliminary work done for the culture of trachoma virus in this inquiry is encouraging but no conclusions can be drawn unless this work has been repeated; the virus grown over several passages, and tested on suitable laboratory animals for pathogenecity. Eight attempts at culture were made and the virus could be propogated for three passages in one series of experiments and over two passages in the other. Further passages were not possible because of bacterial contamination. It is suggested that with improved aseptic technique it may be possible to culture the virus on grafted ocular tissues, over several passages.

The details of the technique, macroscopic and microscopic changes seen in the grafted material, and results of culture experiments have been sent in the form of an illustrated paper to W.H.O. Experts Committee on Trachoma. The paper has been passed for reproduction and translation to the relevant W.H.O. services. A short summary of this paper is also being presented at the Sixth Annual Meeting of Indian Association of Pathologists at Nagpur.

7. Inquiry on virus encephalitis under Dr. P. N. Taneja at the Irwin Hospital, New Delhi.

A detailed report on the epidemic of encephalitis in Delhi was submitted to the Secretary, Indian Council of Medical Research in April, 1955. The present report deals with a review of 70 cases admitted during the period April to October, 1955 and presents an analysis of the follow up studies of 112 cases which survived the acute attack during the period June 1954 to October, 1955.

Routine laboratory investigations were found to be of no specific value in diagnosis. The cerebrospinal fluid was clear in all cases, with raised pressure in about 30 per cent of them. Twenty-seven stool specimens, 28 acute serum specimens and 16 convalescent serum specimens were collected and sent to the Virus Research Centre, Poona for virological investigations. Only one postmortem was done. No specific lesion was observed in the central nervous system, but there was marked fatty metamorphosis of the liver and fatty changes in the convoluted tubules of the kidney. The histological findings, according to Dr. C. G. S. Iyer, are suggestive of some toxic factor in the aetiology of the disease.

During the follow up studies of cases a variety of neurological sequelae was observed. 31·2 per cent of the survivors in the present series showed objective residual manifestation of neurological damage at the time of discharge and 17·8 per cent still continue to show these signs during the follow up period ranging from 2-15 months. 9·8 per cent have shown some form of Parkinsonism. Other sequelae include mental retardation and curtailment of visual and auditory functions.

In pneumoencephalographic study of 11 cases there was positive evidence of varying degrees of cortical atrophy in nine of them.

On the basis of the results so far obtained the authors suggest that possibly more than one factor is at play in the aetiology of this condition.

INDUSTRIAL HEALTH.

1. Industrial Health Research Unit at the All-India Institute of Hygiene & Public Health, Calcutta.

LONG TERM.

1. *Thermal sweating.*

(*a*) *Physiological aspects:*—On lines mentioned in the previous year's report, investigations on various aspects of thermal sweating were continued. As studies on the first subject (B. M.) was concluded last year, experimental determinations were confined to the other two subjects—B.B.S. and M.P.B.—only. Each subject was made to work in a particular climate on a bicycle ergometer against varying frictional loads for one to two hours and observations on concentration of sweat, rectal temperature and skin temperature were taken every five minutes. For each environment and each stress, rate of sweating and oxygen consumption were also estimated. The data are under evaluation for correlation between various factors. A summary of the same is given in Table I.

(*b*) *Physical aspects:*—A detailed paper regarding the utility of electrical conductivity measurement for determination of salt concentration of sweat appeared during this year in the *Indian Journal of Medical Research.* This paper discusses the results of experiments conducted in 1953.

In January this year a short note entitled 'The salt concentration of sweat during exercise in tropical monsoon weather' was published. The complete work has been communicated in a paper to *Indian Journal of Medical Research* for publication. This paper may be summarized as follows:—

(1) The electrical conductivity method of determination of the salt concentration of sweat is extended to volumes of sweat samples as small as 0·03 c.c.

(2) A technique for the micro-sampling of sweat directly from the subject's body is described.

(3) The results of conductivity measurements on consecutive micro-samples of sweat, collected during a single period of sweating from a subject performing exercise at constant rate on a bicycle ergometer in the hot and humid monsoon season in Calcutta are discussed. There was, in general, a progressive increase in the salt concentration of sweat with the duration of exercise.

(4) The micro-sampling technique is compared to the armbag collection method. There is good agreement between the salt concentration of sweat samples obtained by the two methods. Spot samples of micro-quantities of sweat from working subjects are, therefore, good enough for the estimation of the salt concentration of the sweat excreted.

During the series of experiments on sweating conducted in 1954, the electrical conductivity methods for the estimation of the salt concentration of sweat was improved. By using a more accurate chemical technique for determining the chloride concentration of sweat, a new correlation formula between electrical conductivity and concentration of sweat was obtained,

TABLE I.

Details of bicycle ergometer experiments on two human volunteers.

Subject	Date	Dry bulb, °F.	Wet bulb, °F.	O_2 consump. L/ minute,	R.Q.	Average rectal tempera-ture, °C.	Average body tempera-ture, °C.	Met. loss g./ hour	Resp. water loss, g./hour	Sweat loss, g./hour	Average chloride concen-tration of sweat, g./100c.c.	Dura-tion of work, in minutes
M.P.B. ...	17–11–54	83	70	0·776	0·955	37·58	35·89	21·364	48·832	260·5	...	90
	22–11–54	83	68	1·030	1·037	37·49	35·60	38·375	69·962	345·0	0·116	60
	3–12–54	82	72	1·195	1·019	37·47	35·57	41·970	69·738	370·0	0·160	60
Hot room	12–1–55	90	87	1·326	0·848	37·37	36·12	19·677	32·131	533·0	...	60
	2–3–55	94	89	1·244	0·974	38·24	37·31	36·90	35·10	524·0	...	60
B.B.S. ...	10–1–55	75	63	0·717	0·925	36·48	35·21	17·189	40·09	178·0	...	90
	17–1–55	79	69	1·050	0·982	36·92	35·42	32·27	53·39	283·4	...	60
	26–2–55	86	72	1·019	0·854	37·12	35·72	16·45	44·36	384·2	...	60

It has been shown that high correlations of the order of 0·99 exist between the specific electrical conductivity and the constituents of human sweat as shown in Table II:—

TABLE II.

Correlation between the specific electrical conductivity (k) and the chemical constituents of human sweat.

Number	Sp. cond. (k) vs.	Equation	Correlation constant γ
1	Cl MEQ/L	$Cl = 8{\cdot}520 + 8232\,k$	0·983
2	NaCl per cent	$NaCl = -0{\cdot}477 + 47{\cdot}74\,k$	0·990
3	Na MEQ/L	$Na = -4{\cdot}330 + 7758\,k$	0·980
4	Number of ions/L	$N = (-48{\cdot}11 + 97523\,k) \times 10^{20}$	0·990
5	Na+K+Cl MEQ/L	$Na+K+Cl = -8{\cdot}630 + 16153\,k$	0·990
6	Na/K	$Na/K = 0{\cdot}795 + 1336\,k$	0·930

No correlation between specific conductivity and potassium content of sweat was observed. The interdependence of sodium and chloride concentrations in sweat was obtained and this is discussed in the light of previous observations. An inverse relation between the electro-positive ions in sweat (K and Na) is seen to exist. Full details of the study and extensive discussion will be published in due course.

2. *Lung volumes.*

(*a*) *A study on the differential lung volumes of Indian women in health.*—In India, women comprise a large portion of the industrial workers. As unskilled labourers many of them serve in industries where dust is a hazard. It is quite natural that after some years of exposure some of them suffer from chest diseases of various nature, resulting in the impairment of respiratory function. To find out how much incapacitation is done, data on 'norms' of pulmonary function of their healthy counterpart are necessary. With a veiw to formulate the ' norms ' of lung volumes of Indian women, this study was considered necessary. Some amount of work has been done in this country on this aspect but that is limited to only determination of vital capacity and that too is inconclusive. Modern tendency of analysing the efficiency of pulmonary function does not only depend on determination of vital capacity alone, as was thought in the past, but other lungs-compartments also. Elaborate studies on lung volume of men is completed and reported in the previous year. Similar studies on women are in progress. Determinations on tidal volume, inspiratory reserve capacity, expiratory reserve capacity, residual capacity, and functional residual capacity are done in quadruplicate. The subject is first of all instructed as to how she can co-operate to achieve the best results. The runs are done in the morning preferably after a very light food. It is proposed to include twelve subjects from each of the age groups of 15 to 19, 20 to 24, 25 to 29, 30 to 34, 35 to 39 and 40 to 44 for this study.

The data obtained so far are presented in Table III:—

TABLE III.

Differential lung volumes of healthy Indian women.

Group, years	Number of subjects	Age, years	Calculated body surface area m2	LUNG VOLUMES IN RECUMBENT POSTURE AT 'BTPS':									Remarks
				IRC L/m2	TV L/m2	ERC L/m2	VC L/m2	RC L/m2	FRC L/m2	TC L/m2	$\frac{RC}{TC} \times 100$	$\frac{FRC}{TC} \times 100$	
15–19	12	Mean: 17·58 Range: Std.Dev.: ±1·113	1·33 1·13- 1·50 ±0·108	0·87 0·65– 1·14 ±0·171	0·355 0·249– 0·729 ±0·130	0·426 0·252– 0·606 ±0·106	1·71 1·47– 2·28 ±0·229	0·44 0·32– 0·74 ±0·118	0·86 0·62– 1·16 ±0·177	2·14 1.86– 2·76 ±0·308	20·4 14–2– 28·1 ± 4·375	40·3 31·2– 50·6 ±5·406	Complete
20–24	12	Mean: 22·42 Range: Std.Dev.: ±1·349	1·37 1·23– 1·64 ±0·141	1·01 0·64– 1·29 ±0·168	0·279 0·207– 0·357 ±0·041	0·354 0·162– 0·666 ±0·161	1·70 1·20– 2·06 ±0·276	0·54 0·40– 0·68 ±0·936	0·89 0·58– 1·23 ±0·199	2·24 1·76– 2·69 ±0·300	24·2 17·8– 31·5 ±4·195	39·5 32·0– 47·8 ±4·147	Complete
25–29	11	Mean: 27·0 Range: Std. Dev.:	1·47	1·07	0·306	0·391	1·81	0·58	0·97	2·39	24·1	40·3	Incomplete
30–34	7	Mean: 31·6	1·45	0·87	0·339	0·352	1·02	0·52	0·87	2·12	24·9	41·4	Incomplete
35–39	3	Mean: 37·3	1·38	0·91	0·327	0·288	1·58	0·59	0·88	2·16	27·3	40·5	Incomplete
40–44	3	Mean: 41·3	1·53	0·72	0·343	0·205	1·30	0·56	0·75	1·86	28·9	40·0	Incomplete

ERC = Expiratory reserve capacity
TV = Tidal volume
IRC = Inspiratory reserve capacity
VC = Vital capacity

RC = Residual capacity
TC = Total capacity
FRC = Functional residual capacity
Std. Dev. = Standard deviation

(*b*) *A study on the pulmonary functions in thoracic diseases before and after the surgical operation:*—Broadly speaking, pulmonary functions of workers incapacitated by prolonged exposure to dusty environment, are comparable to those of patients suffering from different types of chest diseases. In both the cases, efficiency of the respiratory system is decreased resulting in impairment of pulmonary function. A study to investigate the influence of chest diseases on pulmonary function was started in absence of cases of occupational origin. It was, however, felt that the observations on the experimental group, i.e., clinical cases with chest diseases will have a clear analogy with occupational group so far as this aspect is concerned. Patients suffering from diseases, such as pulmonary tuberculosis, lung abcess, etc., and requiring surgical interventions, such as lobar or segmental resection, thoracoplasty or pneumonectomy were investigated for their lung volumes and pulmonary functions before and after the surgery. Uptil now, pre-operative and post-operative investigations are completed for eight cases. On twenty-seven cases pre-operative investigation is completed, and they are awaiting surgery in the hospital. In Table IV differential lung volumes in various chest diseases are presented. The figures show the extent of incapacitation in pulmonary function with which these patients are handicapped compared to norms which were presented in the previous years. Lowering of vital capacity and maximal breathing capacity to the extent of 20 to 50 per cent and even more is observed in this group as compared to normal values. Residual capacity values increased significantly in a large number of cases. This can serve as a true index of the strain on their pulmonary function. In Table IV*a* a comparison between pre- and post-operative cases in various surgical operations is presented so far as lung volumes are concerned. The details of the study will be reported in the publication later on when the study is completed. After the operation, in some cases, the pulmonary function has improved but in majority of cases it has deteriorated. Improvement is the result of successful removal of only diseased tissue, but whenever some amount of healthy tissue is removed along with the diseased tissue there is deterioration.

3. *Clinical activity:*—The contemplated expansion of the clinical section through collaborating activities of an Industrial Health Clinic at Chetla did not materialize last year as the Urban Health Centre has not yet been opened, as previously understood. Full use, therefore, could not be made of the beds reserved at the Carmichael Hospital for Tropical Diseases for the occupational disease cases.

4. *Health survey.*—Six small foundries, roundabout Howrah, were selected for this survey. The total number of workers in these foundries are about 1,500. The survey is being carried out in two parts; namely (*a*) survey of the working environment, and (*b*) the *x*-ray examination of the chests of the workers. The work is still in progress. The data collected so far can be summarized as follows:—

(*a*) *Environmental survey:*

(*i*) *Dust counts:*—Samples are collected with a Midget impinger at the breathing level of the workers at different operations, such as preparation of the moulds, opening of moulds, pouring and casting operations.

The data collected so far show great variability of the dust counts during the different operations. The counts varied between 2·6 and 162·0

Table IV.

Differential lung volumes (mean±std. dev.) in various chest diseases (pre-operative):

Clinical status	Sex	Age group	Number of subjects	ERC	TV	IRC	VC	RC	TC	FRC	$\frac{RC}{TC}\times 100$	$\frac{FRC}{TC}\times 100$
Pulmonary tuberculosis	M	18–45	12	0·30±0·14	0·28±0·06	0·79±0·35	1·41±0·49	0·50±0·18	1·91±0·57	0·80±0·20	26 51±7·05	42·78±4·04
	F	15–29	5	0·28±0·10	0·27±0·04	0·74±0·22	1·32±0·27	0·49±0·06	1·81±0·29	0·77±0·12	27·34±4·06	43·00±6·46
Bronchiectasis	M	18–45	3	0·41±0·25	0·35±0·11	0·87±0·07	1 70±0 40	0·50±0·14	2·20±0·50	0·91±0·35	22·77±4·56	40·33±6·21
	F	...	...	...	...	...	...	...	...	...	...	...
Lung abcess	M	40–52	4	0·33±0·15	0·32±0·05	0·88±0·25	1·58±0·40	0·54±0·14	2·12±0·48	0·87±0·24	25·70±5·15	40·43±4·45
	F	16	1	0·19	0·24	0·97	1·40	0·38	1·78	0·57	21·10	32·00

ERC=Expiratory reserve capacity.
TV =Tidal volume.
IRC=Inspiratory reserve capacity.
FRC=Functional residual capacity.

VC=Vital capacity.
RC=Residual capacity.
TC=Total capacity.
Std. Dev.=Standard deviation.

TABLE IV*a*.

Comparative differential lung volumes (average) before and after various surgical operations.

Nature of surgical operation	Sex	Age group	Number of subjects		ERC	TV	IRC	VC	RC	TC	FRC	$\frac{RC}{TC} \times 100$	$\frac{FRC}{TC} \times 100$
Pneumonectomy	M	18–25	2	Pre	0·26	0·26	0·80	1·42	0·50	1·92	0·78	26·1	40·0
				Post	0·28	0·36	0·50	1·20	0·62	1·82	0·90	33·5	49·3
	F	...	...		...	...	...	...	...	...	...	...	...
Thoracoplasty	M	17–43	2	Pre	0·25	0·23	0·83	1·40	0·72	2·12	0·97	33·8	46·8
				Post	0·26	0·23	0·35	0·88	0·28	1·16	0·56	25·6	47·3
	F	...	...		...	...	...	...	...	...	...	...	...
Segmental and lobar resection	M	28–52	2	Pre	0·54	0·30	1·30	2·20	0·48	2·68	1·02	18·2	38·2
				Post	0·32	0·41	0·54	1·30	0·46	1.76	0·78	25·9	44·0
	F	16–22	3	Pre	0·27	0·24	0·80	1·32	0·48	1·79	0·74	26·5	41·5
				Post	0·22	0·28	0·62	1·15	0·48	1·63	0·70	29·3	43·1

Pre=Pre-operative. Post=Post-operative. ERC=Expiratory reserve capacity. TV=Tidal volume. IRC=Inspiratory reserve capacity. VC=Vital capacity. RC=Residual capacity. TC=Total capacity. FRC=Functional residual capacity.

million particles per cu. ft. of air, the opening of the moulds being the dustiest.

(*ii*) Chemical analysis of the air-bourne dusts was made for their free silica content, by the hydrofluosilic-acid method of Durkan. The silica content of the different dust samples, analysed so far, range from 12 per cent to nearly 30 per cent.

(*iii*) *Environmental warmth:*—Data on environmental warmth have been collected in these foundries near the various operation sites and at different times of the day. The data collection have not yet been completed. It was seen that near the cupolas during working, globe thermometer readings up to 38° F. higher than the dry-bulb readings were obtained. In the sheds for drying the moulds also, large differences in the dry bulb and Globe thermometer readings were obtained.

(*b*) *X-ray examination of the chests of the workers:*

So far 102 workers from factory I, 109 workers from factory II, 106 workers from factory III and 289 workers from factory IV have been *x*-rayed and the skiagrams studied.

Of these 606 workers, 14 cases with tubercular lung infiltration were detected, with an incidence of 2·3 per cent. The chest *x*-rays of 14 other workers from the above group show abnormalities suspected to be due to the effects of dust. Periods of service in the foundry of these workers range from 10 to 35 years with an average of 23·5 years. These workers will be followed up at regular intervals to arrive at a more definite conclusion.

5. *Industrial tension:*

(*a*) *Workers' attitude survey:*—This study included three hundred and ten workers of various categories below the supervisory level in an engineering industry at Calcutta. Human relations interview technique was followed to assess the existing morale in the plant. This survey revealed the amount of tension prevailing in the different departments of the factory. A five-point weighted attitude scale including six principal factors was used to assess the workers' morale. It can be said from the result obtained that pay has some influence on the workers' morale but that is not the only criterion controlling workers' attitude disposition. It was also observed that workers appreciated the nonfinancial benefits offered to them. A few points of grievances, such as increment, bonus, canteen, optimum working space, safety measure, medical service, personal relations between different levels of personnel, etc., were noted. It was further observed that the union influence was very strong though it was not recognized by the management. The workers demanded better and sympathetic treatment from the management and wanted that their definite status should be recognized.

Table V shows the percentage of workers distributed in the five-point attitude scale with reference to their respective total attitude scores. Only one per cent of the workers interviewed belonged to the neutral attitude group which suggests that the workers of this factory have definite attitude direction irrespective of their educational backgrounds.

TABLE V.

Showing the percentage of workers distributed over the five-point attitude scale.

Total attitude	Per cent
(*a*) Most favourable	13
(*b*) Favourable	33
(*c*) Neutral	1
(*d*) Unfavourable	40
(*e*) Most unfavourable	13

Mean attitude score was determined to be = 74·27 ± 8·96.

Table VI summarizes the important findings regarding the relationship between the average scores in the factors comprising the attitude scale and the corresponding total average attitude scores. All the correlation values were found highly significant.

TABLE VI.

Summary of the relationship of the average scores in the attitude factors to the total attitude scores.

Factors	Co-efficient of correlation = r	P Er.
(*a*) Attitude towards the management ...	0·4696	0·0299
(*b*) ,, ,, the job	0·6002	0·0245
(*c*) ,, ,, fellow workers	0·5528	0·0266
(*d*) ,, ,, supervisors	0·4733	0·0298
(*e*) ,, ,, national government ...	0·4193	0·0316
(*f*) ,, ,, the society	0·1839	0·0371
(*g*) ,, ,, the trade union	0·2664	0·0356

The percentage distribution of workers in a five-point attitude scale, in the various departments of an engineering industry in Calcutta is given in Table VII. The findings of this study, especially, the amount of tension existing in the different sections of the factory were corroborated with the estimation of the management and the labour department. The tension was found maximum in the sections F, E, and G.

This project also included the application of psychological principles to orient industrial personnel's attitude disposition. This programme would help the maladjusted workers to ease-off their personal difficulties and thus promote the mental health of the industrial workers. The work of this first part of the programme is completed.

TABLE VII.

Percentage distribution of workers in the attitude scale in the various departments of an engineering industry.

Department	Most favourable, per cent (*a*)	Favourable, per cent (*b*)	Neutral, per cent (*c*)	Unfavourable, per cent (*d*)	Most unfavourable, per cent (*e*)
A	26·7	33·3	...	40	...
B	16	37·6	7	37	8·7
C	14·3	32·7	...	36·7	16·3
D	10	34	...	38	18
E	...	26·9	3·9	53·8	15·4
F	...	12·5	...	75	12·5
G	4·2	25	...	45·8	25

(*b*) *Supervisors' attitude survey.*—Twenty supervisors of the same factory were interviewed in camera. A suitable attitude scale was constructed and used to assess the supervisors' morale. The analysis of the data has been taken up and the results will be duly communicated.

(*c*) *Supervisors' orientation programme.*—The Supervisors' Orientation Programme is a part of the research project on industrial tension. In this connection, the supervisors of a particular department of the factory are undergoing an 18-hours orientation course. The object of this programme is to determine the effective value of supervisors' training in human relations on the morale of the industrial personnel. The results of this orientation programme will be studied by the subsequent attitude survey of the same groups of workers and supervisors.

(*d*) *Women workers' attitude survey:*—Women workers in India are mainly engaged in mines, jute and cotton industries and only a very limited number in mechanical and technical jobs. Out of the total workers engaged in factories in West Bengal only 8·3 per cent are women workers. The object of the present study was to determine the attitude disposition of a group of 55 women workers engaged in semi-skilled job in a factory manufacturing electrical goods near Calcutta. The significance of this study was that this survey was limited within a group of displaced women workers. The mental disposition of this women folk was a bit different from ordinary working people in factories. An unfavourable orientation towards the national government was noted. This may be due to their insecure socioeconomic condition and the difficulty in accommodating with the new life situation. The family-tradition and prejudice were found to influence the behaviours of these women workers in the factory which ultimately have given rise to mental unrest. The inhibiting factors together with other changes in the social life were responsible for their frustrated attitude towards the Government and the industry. Possibly, this is the first attempt to make a preliminary over-all attitude survey of women workers engaged in Indian industry. It is to be noted that the present data are not sufficient to draw any definite conclusion. Further comprehensive study in this line is needed for the purpose.

6. *Study on manual dexterity and steadiness of skilled workers:*—This pilot study included 22 skilled workers of a particular department of an engineering industry near Calcutta. The main object of this study was to determine the reliability and validity of the manual dexterity and steadiness tests and to find out the predictive value of these test scores so far as the individual's job success was concerned. The effects of precision job on the physiological system of the workers were also studied.

The average dexterity test score was determined to be 367·45±36·4. Similarly, the average steadiness test score was found to be 0·12±0·04. The correlations between the steadiness test and dexterity test scores and foreman's ranking and dexterity test scores were 0·41 and 0·42, respectively. On the other hand, the average systolic and diastolic blood pressures of this group of workers were found to be 114·5 and 66·5 mm. Hg. No case of abnormal blood pressure was noted. The average pulse rate before and after experiment was 74 and 80 per minute, respectively. Attempts were also made to study the influence of precision job on the blood pressure and pulse rate of the individual personnel. No significant variation of blood pressure or pulse rate was noted.

Significant correlations between the supervisor, tester and foreman's ranking and the actual test scores were observed which proved the validity of the dexterity and steadiness tests. These tests will be applied on a larger group of skilled workers to confirm the findings of this pilot study.

7. *Air pollution:*—A great interest in the problem of atmospheric pollution has occurred recently all over the world. Mellon Institute of Industrial Research in Pittsburgh, U.S.A. investigated this problem from 1911 to 1914 in that city. The British Government Department of Scientific and Industrial Research has been systematically studying this problem since 1927. Interest in this subject has further been stimulated by the dramatic episodes of air pollution, associated with loss of life and increased incidence of illness, such as the Meuse Valley incident in 1930, the incident at Donora in 1948, the hydrogen sulfide tragedy in 1950 of Poza Rica and the heavy smog of London in 1952.

No effort has been made in India uptil now to study this problem. A casual visit to a big city, such as Calcutta, specially during the winter months will immediately convince one about the importance of this problem. The city appears at that time to be covered with a veil of smoke, irritating to the eyes and nose and causing difficulty in visibility.

It is with a view to study the exact condition of the air in Calcutta so far as the different pollutants are concerned, that this study has been initiated. The study is now in a preliminary stage and its scope is expected to expand considerably next year on the basis of information collected in this preliminary study. This study is now divided in two groups: (*a*) dustfall collection, and (*b*) estimation of gaseous constituents in the air.

(*a*) *Dust-fall collection:*—Glass-jars of the dimension of approximately 6″×8″ have been placed at different parts of Calcutta on the house roofs. The jars contain a small amount of water and a little of 0·5 per cent $CuSO_4$ solution to prevent the formation of moulds and algæ. At the end of every calendar month the jar is replaced by a new one and its contents are brought to the laboratory for analysis. The contents of the jar are filtered through a filter paper and the soluble and insoluble portions dried up, the sum of them gives the amount of dust fall per month in an area represented by the cross-section of the jar. From this is calculated the total amount of dust-fall per month per sq. mile of Calcutta. Table VIII gives a summary of the average monthly dust-fall per sq. mile in tons during the last few months in Calcutta.

TABLE VIII.

Average dust-fall per month expressed in tons per square-mile.

Month	March	April	May	June	July	August
Range	64·36 ...	67·16 (65·62–68·69)	70·88 (41·09–90·55)	97·69 (76·84–121·08)	39·47 (28·70–49·75)	33·49 (24·52--43·79)

The dust is then put in a porcelain crucible and heated in a burner to drive away all the volatile matters, and also to burn away the carbonaceous materials; the ash left gives an idea of the mineral content of the dust. Table IX gives an analysis of the dust so far as its ash contents and fixed carbon and volatile matters are concerned.

TABLE IX.

Analysis of the dust from the dust-collectors.

Month	Amount of dust insoluble in water, per cent	Amount of dust soluble in water, per cent	Percentage of fixed carbon including volatile matters	Percentage of ash content
March ...	82·69	17·31	28·93	53·75
April	65·48	34·52	11·53	53·95
May	88·49	11·51	26·21	62·29
June	73·61	26·39	18·88	54·74
July	67·59	32·41	19·96	47·63
August ...	66·50	33·50	23·59	42·91

(*b*) *Estimation of gaseous constituents*:—There are quite a big number of gaseous constituents in air besides elemental nitrogen and oxygen which are supposedly present in pure air. At the present stage of our study it has been possible to estimate only three gases, namely SO_2, NH_3 and NO_2. These gases are present in the atmosphere at such a small concentration that special sampling devices and micro-techniques are necessary for their analysis. *Estimation of SO_2*:—45 cu. ft. of air are sampled in a standard impinger containing a known amount of alkaline-iodine solution at the rate of 1 cu. ft. per minute. After the sampling the iodine solution is titrated against a standard sodium thiosulphate solution. Similarly, a blank titration is made using the same volume of alkaline iodine solution. From the difference of the two is derived the amount of SO_2 present in the air. Table X gives an indication of the concentration of SO_2 during the past few months in Calcutta atmosphere. The estimation has been made twice every week.

Estimation of NH_3:—Samples are collected in a Midget impinger containing 0·002 N sulfuric acid. The acid is then titrated with standard alkali. From the amount of sulphuric acid neutralized by NH_3, the concentration of the latter is calculated. The results are given in Table X:—

TABLE X.

Monthly average of gaseous contaminants in the atmosphere.

Months	Sulphurdioxide, ppm.	Ammonia, ppm.	Oxides of nitrogen expressed as NO_2, ppm.
July	0·0973 (0·0704-0·1330)	3·8	...
August ...	0·0973 (0·0488-0·0873)	1·51 (1·15-2·45)	0·15
September ...	0·027 (0·020-0·040)	2·01 (0·92-3·20)	0·025 (0·012-0·048)

Figures in the brackets indicate the two limits of values found.

Estimation of NO_2.—NO_2 combines with a mixture of alphanaphthylamine and sulphanilic acid in glacial acetic-acid solution to produce a pink colour. The intensity of this colour forms the basis of estimation of NO_2 in air by using a Beckman spectrophotometer at a wave length of 522 mμ. Table X gives an idea about the concentration of NO_2 in Calcutta air.

8. *Experimental pneumoconiosis.*—One dozen monkeys were maintained in the batching shed of a jute mill near Calcutta. They were exposed to the dusty environment during a period varying from seven months to slightly more than three years. The average duration of their stay was about one year nine and a half months. All these monkeys were *x*-rayed for excluding the presence of lung lesions, such as infiltration, before putting them into the jute mill.

The histopathological appearances of the lungs of six monkeys have been studied so far in co-operation with the Pathology Department, Calcutta Medical College. The lung sections were stained with hæmatoxylin and eosin as well as with Van Gieson stain in order to study any increase in the connective tissue of the lung.

The more common findings were varying degrees of engorgement of the blood-vessels, some mild hyperplastic changes in the bronchiolar epethelium as well as scattered round cell infiltration in the sub-epethelial layers. Emphysema was also almost constantly present but was never marked except in one monkey which before death had been suffering for some time from paroxysmal dyspnœic attacks. Post-mortem findings in the last case also showed large bullous emphysemata. In two others, one of which died and the other sacrificed while suffering from temperature, dyspenœa and cough, areas of consolidations were present in the lungs.

Pigments were mainly present round the perivascular and peribronchial connective tissue or in macrophages here and there within the alveolar lumen. No other extraneous matter, such as long fibrous material resembling jute, was found in any lung section, except the dark-brown granular pigment which were present in fair amounts and were distributed as indicated.

Evidence of any increase in the connective tissue was lacking in any of the lung sections. It does not appear from what has been observed so far, that the inhalation of jute dusts for prolonged periods produce any specific changes of the lung. The histopathological study of the lungs of the remaining monkeys are still in progress and is expected to be completed shortly.

SHORT TERM.

1. *Illumination.*—(*a*) A complete study of the lighting conditions in 12 factories belonging to six typical industries in Calcutta has been made. A summary of the findings was reported last year. The results of this investigation were analysed and a report incorporating the recommendations was sent to the management of the factories. The results and conclusions have been communicated in a paper entitled 'A preliminary investigation of lighting conditions in some typical industries in Calcutta' to *Indian Lighting* for publication. A summary of the same is given below.

Summary.—The lighting conditions in twelve factories belonging to six different industries in Calcutta, were studied in detail both during day and night, on a job-wise basis. In general, say for a few exceptions, the level of illumination, artificial, natural or both prevailing in these factories is subnormal in comparison to standards prescribed in Western countries. Only 34 per cent of the points during day and 16 per cent points during night had illumination that may be considered adequate for the operation performed at the point. While the above remark is true to the quantity of illumination, it is no less applicable to the quality of the lighting, which was adjudged on the basis of factors influencing ease of vision. An extensive discussion of the common defects in the illumination in these factories is given.

(*b*) As proposed in the last year's report, a draft scheme for the study of:—

(*i*) Visual fatigue in industrial workers.

(*ii*) Illumination requirements for industrial operations have been prepared. In this scheme the importance of conserving and improving the visual capacity of industrial workers has been emphasized. Methods of studying the fatigability of the eyes and the fixation of illumination standards are briefly described. The economical feasibility of this project is pointed out and attention is drawn to the offer of Messrs. Philips Electrical Co. (India) Ltd., for the loan of equipment necessary for these investigations.

2. *Radiation hazards.*

(*a*) *Physical environment survey.*—In continuation of the report presented last year on this project, the physical environment survey of 15 medical *x*-ray installations in Calcutta has been completed, along the lines described before. During this year the film badge technique was not, however, used due to its limitation. From (*i*) the evaluation of the status of the general environment in these *x*-ray establishments in the light of the recommendation of the International Commission of Radiologial Protection, (*ii*) the evaluation of status of the *x*-ray source and protection practice adopted by the personnel, and (*iii*) the measurement of the radiation intensity at various places, suitable recommendations and safety regulations for ensuring the protection of personnel have been made. A brief report of the environmental survey and the estimation of the secondary radiation by physical methods is given below.

The earliest recommendations put 1·0r (1000 mr.) as the maximum permissible limit of radiation exposure per week of five working days. Subsequently, the maximum permissible limit has been graded down to as low as 0·3r per week inside the basal layer of the epidermis. A maximum limit of 0·5r per week in air has, however, been adopted in this present study. This limit ensures that the personnel working with *x*-rays have the least chance of developing skin changes, which might in the long run develop into dangerous cancer, or persistent blood changes which might ultimately give rise to leukæmia.

In this pilot study fifteen medical *x*-ray installations, one of which was therapeutic and the rest diagnostic, were investigated for possible *x*-ray hazards.

The physical estimation was done by two methods utilizing the photographic and ionizing properties of *x*-rays, respectively. In the former method the blackening of dental *x*-ray films kept in fixed place for 7 to 12 days was measured. The record of exposure received by each film was kept on a card along with its distance from the *x*-ray tube. This photographic method was used in six diagnostic installations only, and subsequently discarded for want of accuracy.

The ionization method is, however, more reliable for the assessment of the hazard. A Stephens pocket dosemeter with a maximum range of 200 milliroentgens was used for these measurements. The dosemeter was put at one meter distance from the tube and readings taken after a definite time of exposure to the *x*-ray tube. As many repetitions as possible were made under actual working conditions, i.e., with the patient in position. The amount of radiation and, therefore, the ionization, depend on the distance from the tube and the time of exposure.

The average time of exposure per week for estimating the hazard was computed from records specially kept for a few days. This was only approximate and had to be taken since the records of these hospitals do not show the details of each exposure, i.e., time of exposure, kilovoltage used, etc. The effect of the kilovoltage and milliamperage used on radiation intensities was not taken into account in the present study.

Simultaneously, the following environmental factors were also observed:—

1. Ventilation.
2. Lighting.
3. Temperature and humidity.
4. Layout of equipment.
5. Electric wiring.
6. Position of master switch.
7. Type and condition of *x*-ray tube.
8. Presence of secondary scatterers.
9. Distance of personnel.
10. Knowledge of radiation hazard.

Special note of all protective devices and protection practices as well as the malpractices were noted.

The density of blackening of the film is proportional to the amount of radiation received. The density of blackening is measured by the transmission of light, measured with a simple photosensitive device. The accuracy of the present method is not very high. Only comparisons of radiation intensities are possible. Ratios of the radiation intensities were calculated assuming the film with the lowest opacity as unity. It is to be noted that this ratio holds only for cases where the opacity is not complete. In the cases where blackening is complete, the radiation intensity may be much more than warranted by the value. Further films are to be exposed for a much shorter period, for getting a more reliable value. The ratio of intensity in the six installations obtained by photographic and ionization methods show a first order of agreement. The results are given in Table XI.

The ionization method, however, gives the value of radiation intensities directly in milliroentgens. The values are, as a rule, more reliable. Measurements were taken at a standard distance of one meter from the *x*-ray tube in order to make comparison of various installations possible. Assuming a maximum permissible limit of 500 mr. per week the total exposure time with each tube for this distance has been given in the table. To estimate the hazard, the radiation received by the operator, if he stands near the control board has been calculated for the average weekly working time for each installation. The inverse square law for radiation attenuation has been made use of. The values are also given in Table XI. Measurements were made at the operator's position also in installations where there was sufficient exposure in a day to give an appreciable reading. The agreement between the calculated and observed values serves as a check of the accuracy. It is seen from these values that the exposure received is well below the safe limit in all except one case. In this exceptional case, a measurement at the actual spot was made and a high value was obtained. It was observed that the lead-lined door separating the operator from the tube was not kept closed, as should have been done. A possible hazard exists here, which may, however, be eliminated by keeping the door closed. Reading when the door is kept closed is only 50 mr. per week which is well below the maximum permissible limit.

The analysis of protection practices in these installations indicates that protective devices are not popular in the majority of cases, although most of the installations are provided with them. Knowledge of radiation injuries and practice of protection methods are also insufficient amongst personnel other than the radiologists. The number of no's under the column

TABLE XI.

Summary of environmental monitoring in x-ray *installations.*

Installation	IONIZATION DOSEMETRY				FILM DOSEMETRY	USE OF PROTECTIVE DEVICES			Maximum working time permissible, minutes	Special remarks, if any
	At 1m mr./minute	Weekly exposure received by operator		Relative ratio of intensities	Relative ratio of intensities	Lead screen	Apron	Gloves		
		Obs. mr.	Cal. mr.							
1	2	3	4	5	6	7	8	9	10	11
A_1 and A_3	12·20	...	11·13	1·00	1·00	No	No	No	40·00	...
A_2 ...	26·90	15·36	21·12	2·20	...	Yes	Yes	Yes	18·60	This unit is the best protected, least hazardous; this is so because the hazards of roentgen therapy which are high are realized.
B ...	83·40	...	80.55	6·84	3·92	No	No	No	6·00	Seating accommodation for the non-technical staff should not be provided in *x*-ray room.
C ...	151·30	...	82·70	12·40	15·30	No	No	No	3·30	...
D ...	81·30	...	27·70	6·68	5·81	No	No	No	6·14	The door leading to the nurses' quarters from the *x*-ray room needs some protection.

TABLE XI. (Contd.)

Summary of environmental monitoring in x-*ray installations.*

Installation	IONIZATION DOSEMETRY				FILM DOSEMETRY	USE OF PROTECTIVE DEVICES			Maximum working time permissible, minutes	Special remarks, if any
	At 1 m mr./minute	Weekly exposure received by operator		Relative ratio of intensities	Relative ratio of intensities	Lead screen	Apron	Gloves		
		Obs. mr.	Cal. mr.							
1	2	3	4	5	6	7	8	9	10	11
E ...	106·30	...	28·14	8·71	7.93	No	No	No	4·70	A nurse trained to assist the radiologist in taking *x*-ray pictures is essential.
F ...	744·70	660·00	618·90	61·04	17.20	No	No	No	0·67	By closing the lead door separating the operator from *x*-ray room, the radiation (secondary) can be brought down to a safe level.
G_1 ...	97·40	118·13	116·00	7·98	...	No	No	No	5·00	A hazardous intensity is detected near the door. Patients and others are forbidden to stand there.
G_2 ...	380·40	224·38	266·67	31·18	...	Yes	No	No	1·30	An area 1·5 m. round the tube is dangerous.
G_3 ...	1·31	134·13	254·06	·11	...	Yes	No	No	380·00	The forward beam is not properly cut off, and contributes high intensities to G_1.

TABLE XI—(*Concld.*)

Installation	IONIZATION DOSEMETRY				FILM DOSEMETRY	USE OF PROTECTIVE DEVICES			Maximum working time permissible, minutes	Special remarks, if any
	At 1/m mr./minute	Weekly exposure received by operator		Relative ratio of intensities	Relative ratio of intensities	Lead screen	Apron	Gloves		
		Obs. mr.	Cal. mr.							
1	2	3	4	5	6	7	8	9	10	11
H_1 ...	133·30	55·04	40·00	10·93	...	No	No	No	3·75	A high intensity of radiation is present in the forward direction.
H_2 ...	22·76	82·80	80·98	1·87	...	No	No	No	22·00	...
I ...	72·90	68·40	60·74	5·98	...	No	No	No	7·00	Technicians holding patients during radiography receive hazardous *x*-ray exposures.
J ...	1·03	365·40	132·30	0·08	...	No	No	No	485·00	An unprotected person, if present for an extended period within a distance 1·5 m. from the tube, is likely to be exposed to a high weekly radiation.
K ...	19·67	17·48	21·30	1·61	...	No	No	No	25·40	The state of this installation is very poor. It is necessary to renovate the system entirely.
L ...	2·20	31·60	4·96	0·18	...	No	No	No	225·00	Aprons and gloves are to be provided.
M ...	4·78	...	9·75	0·39	...	No	No	No	104·00	...

m=meter. mr.=milli-roentgens.

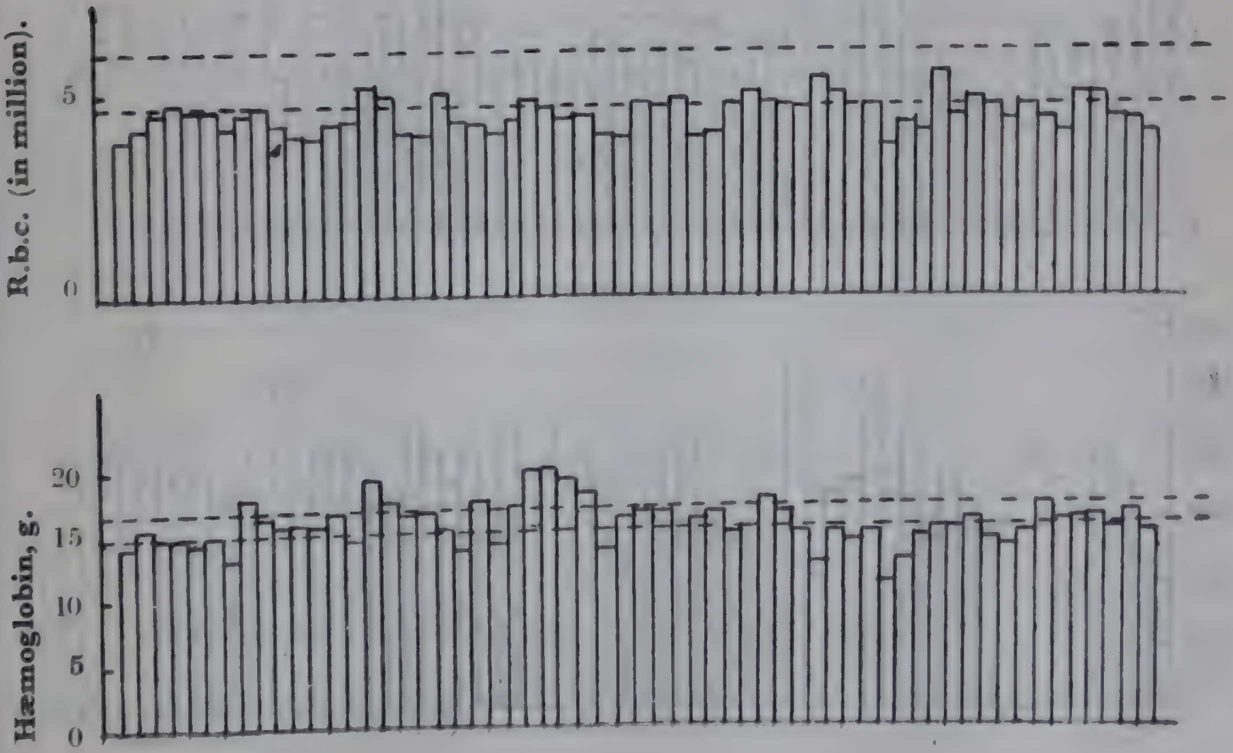
use of protective devices in Table XI is quite impressive. Protection practice was satisfactory only in installation A_2, a therapy unit. This must be because the hazards of roentgen therapy are well realized. It was, however, evident that most technicians and some radiologists even, have no hesitation at all in exposing themselves to direct radiation or working close to the patient while exposure is going on. It is, therefore, probable that they receive much more radiation than is justified by our measurements.

It is surprising that many cases of radiation damage were not apparent amongst the personnel, considering the fact that the personnel are not shy of a few direct exposures. This is primarily because the pressure of work is not high. In the installations where there is maximum exposure per week, secondary radiation is low (installations A_1, G_1, G_3) or there are many assistants who follow a rotation system (installation G_1, J). Whereas in the installations where secondary radiation hazard is high, fortunately, the number of pictures taken in a week is few (installations C, E). In installation F, a mobile unit, the hazard is maximum, but the work has been intermittent so far with long intervals of inactivity due to various causes. The medical examination, therefore, did not reveal any evidence of injury. It is imperative that protection practices should definitely be adopted if these installations go beyond the maximum exposure time per week indicated in Table XI under the prevalent working conditions.

FIGURE 1.

Horizontal interrupted lines represent the average normal range.

(Each vertical column represents the value for an individual subject)

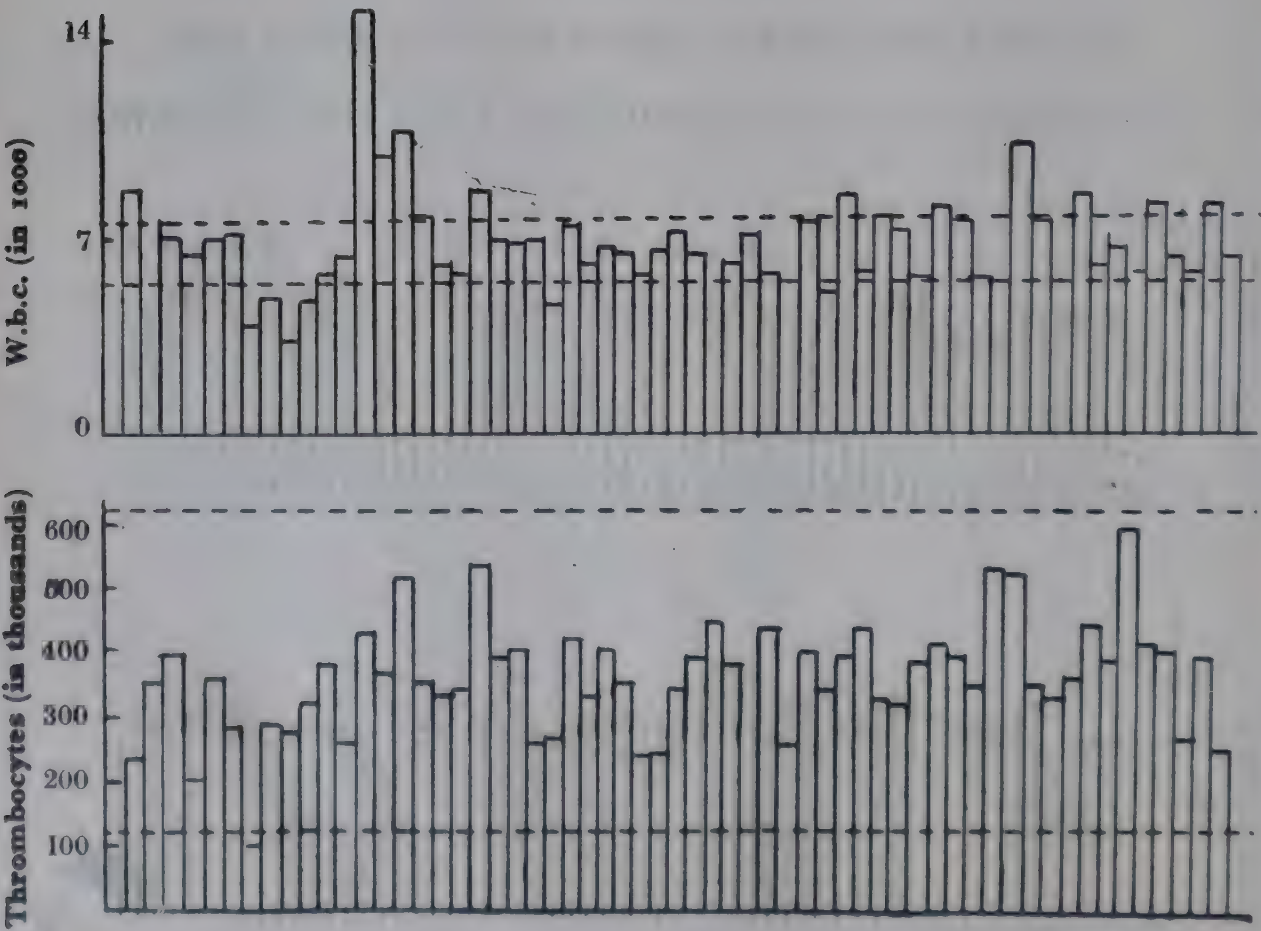


(*b*) *Clinical and hæmatological examinations:*—In connection with the survey of the radiation hazards, blood samples of 60 persons from the different *x*-ray clinics, surveyed were taken. Of these, 32 were medical personnel, 16 technicians, 9 peons and 3 nurses. Total counts of r.b.c., w.b.c., platelets, hæmoglobin and differential counts were performed on these persons. Values for r.b.c. and hæmoglobin, w.b.c. and platelets are shown in Figures 1 and 2. It will be seen from these Figs. that major departures from the normal range are not present. Forty of the above personnel were also interviewed and clinically examined. Their periods of service in the *x*-ray departments varied between four months and 28 years with an average of slightly more than six years. Only in one case healing radiation dermatitis of the terminal phalanges of both hands was observed. This laboratory attendant was required to hold casettes with his bare hands against the chests of patients in the absence of a casette holder. At the time of examination, when the condition was detected, the person had just joined after six months of leave and a casette holder purchased. No other definite signs of radiation-injury were observed in any of the other personnel.

FIGURE 2.

Horizontal interrupted lines represent the average normal range.

(Each vertical column represents the value for an individual subject)



3. *Seasonal variation of basal metabolic rate:*—It has been reported in the previous year that a pilot study on this problem was undertaken. On experiences gained in that study, an elaborate programme on the actual study was chalked out. It might be mentioned here that researches on energy output at basal level of Indian population is necessary to formulate the standards of metabolic cost in industries. Unless correct values are obtained on the basal condition, attempts to measure the daily energy output of any worker will be a failure. Researches on those lines in India have failed to define the standards, so it was considered necessary to work out this problem on a correct scientific basis.

Two subjects from each age group of 20 to 30, 30 to 40 and 40 to 50 were selected from middle class Bengali population after strict examination of their physical conditions, habits and dietary practices. Any factor which may influence the b.m.r. was carefully considered and attempts were made to eliminate them as far as possible. On each subject b.m.r. tests in duplicate were run each week. Usually, the best was undertaken 14 hours after the last meal and after eight hours of undisturbed sleep. The subjects slept in the laboratory on the previous night. Urinals and bedpans were provided to cut short any voluntary movement. Tests were run usually between 7 a.m. to 9 a.m. in a quiet room. Elaborate checks on the environmental condition of the climate and pulse rate, blood pressure and oral temperature of the subject were taken and efforts were made to make the subject maximally comfortable during the run.

This study is in progress from March, 1955 and will be completed in February, 1956. The data collected so far in this study are presented in Table XII.

TABLE XII.

Seasonal variation of basal metabolic rate in adult Indian males.

MONTHS:		MARCH						APRIL					MAY				
Name	Age group	BSA M^2	Number of days	P.R. /Min.	B.P. mm. of Hg.		BMR Kcal/ M^2/Hr.	Number of days	P.R. /Min.	B.P. mm. of Hg.		BMR Kcal/ M^2/Hr.	Number of days	P.R. /Min.	B.P. mm. of Hg.		BMR Kcal/ M^2/Hr.
					Sys.	Dia.				Sys.	Dia.				Sys.	Dia.	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
ABS	40–47	1·76	3	64	120	85	35.9	5	63	119	83	35·2	4	67	113	82	36·5
BBS		1·82						4	62	118	79	34·5	2	66	108	70	35·9
SNG	30–35	1·72						4	77	156	103	33·9	4	80	161	113	37·2
HS ...		1·66						4	71	106	55	31·7	4	73	113	64	30·6
MPB ...	20–25	1·56	3	64	113	74	32·5	4	67	108	71	30·7	4	68	113	71	30·5
BPC ...		1·43	3	72	102	70	35·0	5	67	99	66	32·5	4	63	101	58	32·9
MONTHS:		JUNE						JULY					AUGUST				
ABS ...	40–47	1·76	4	65	121	85	37·1	5	65	116	86	34·9	4	60	111	85	34·5
BBS ...		1·82	4	59	116	81	34·6	4	57	112	82	36·6	4	59	116	78	36·6
SNG ...	30–35	1·72	4	77	155	110	34·4	5	78	154	104	35·9	4	75	150	104	36·6
HS ...		1·66	4	69	107	63	31·2	5	72	104	65	30·4	4	70	104	62	32·5
MPB ...	20–25	1·56	4	61	109	70	36·6	5	64	104	71	31·4	4	66	110	69	34·9
BPC ...		1·43	4	64	96	53	31·1	5	66	97	57	34·5	4	66	99	63	36·3

BSA = Body surface area
B. R. = Pulse rate
B. P. = Blood pressure
Sys = Systolic
Dia = Diastolic
BMR = Basal metabolic rate
Kcal = Kilo calories

2. Inquiry into the effect of sewage treatment and excreta disposal methods on intestinal parasites under Dr. T. R. Bhaskaran at the All-India Institute of Hygiene & Public Health, Calcutta.

The work so far carried out relates to standardization of a suitable technique for the isolation and quantitative estimation of tubercle bacilli from highly contaminated sewage and sludge samples. Materials obtained from the Jadavpur Sanatorium Sewage Treatment Plant are being examined for this purpose. Aliquots of raw sewage and effluents were collected over a period of about eight hours and processed. Morning samples were preferred for obvious reasons.

About 250 c.c. of the pooled material is processed in the laboratory either on the same afternoon or stored at a temperature below 4°C., when necessary, up to a period of three to four days. Each sample is thoroughly shaken with glass-beads and allowed to settle at room temperature for one hour in order to sediment coarser particles. The supernatant is centrifuged at 4,000 r.p.m. for 30 minutes. The deposit is then pre-treated with acid or heat to eliminate secondary organisms as far as possible. The deposit is divided into two parts; one treated with six per cent H_2SO_4 with subsequent neutralization and the other heated at 50°C. for 30 minutes. Some of the heat-treated samples were also treated with penicillin solution (10 units per c.c.).

Smears from centrifuged deposits before or after the above treatment were stained by Ziehl-Neelsen's method and acid-fast bacilli noted in some cases. It is, however, difficult to categorise them as tubercle bacilli because of the possible presence of other saprophytic acid-fast organisms in materials of this nature.

The processed materials were inoculated on Lowenstein-Jensen's medium in McCartney screw-capped bottles and observed over a period up to eight weeks. Media inoculated with the heat and penicillin-treated material showed slightly less contamination. Even in contaminated cultures, tubercle bacilli could, however, be demonstrated in the water of condensation. Work on re-treatment of this material and subsequent sub-culture is in progress. A start has also been made to improve the Lowenstein-Jensen's medium by incorporating penicillin.

It has not been possible to inoculate guinea-pigs with the processed samples uptil now. A stock of the animals for this purpose is now ready for the same.

By adopting the foregoing technique of processing and culture it has been possible to isolate the tubercle bacilli from sewage. There is a need of further laboratory controlled experiments to improve the media.

3. Industrial wastes disposal and water pollution research unit under Dr. T. R. Bhaskaran at the All-India Institute of Hygiene & Public Health, Calcutta.

SUGAR WASTES.

A survey of the sugar factories in Uttar Pradesh and Bihar has been carried out with a view to determine the quantity of different types of liquid wastes produced, their chemical characteristics and difficulties in their sanitary disposal. The survey-schedule form used for the purpose is appended with the note. Out of the 76 sugar factories in U.P. and 47 in Bihar, only eight representative factories were chosen for detailed survey; and these were so selected as to give a cross section of the whole industry from the point of view of waste disposal problem. The factories were classified into three groups. Group I consisted of factories with a daily crushing capacity of 1,300 tons and over, group II consisted of factories between 1,000 and 1,300 tons per day and group III with crushing capacity below 1,000 tons per day. Representative factories in each of these groups were included in the survey.

Data obtained from the sugar factories surveyed in U.P. and Bihar are presented in Table I.

The results show that the wastes have a pH range from 6·7 to 7·5. The suspended solids and oxygen-consumed values range from 300 to 1,440 ppm and 840 to 980 ppm, respectively. The wastes are turbid in nature and putrify on storing for some time.

It was also observed during this survey that most sugar factories are located in areas where there are no big rivers or water courses available for satisfactory disposal of these wastes. Only a few factory owners utilize the effluent for irrigation purposes, while the majority of them let out the waste waters, including sanitary wastes either on open land or into the small seasonal nallahs where these wastes create insanitary conditions. Lack of proper drainage facilities inside the factory premises also lead to stagnation of waste and nuisance in certain localities.

Another important factor noticed during the survey was poor housekeeping in many of the factories. The volume of the waste waters was unnecessarily increased by allowing condenser water to mix with the factory effluent, resulting in excessive quantities of diluted effluent to be dealt with. This situation, however, could be improved by providing separate channels to take away the excess dilution water which is comparatively clean. It was also seen in many factories, where care is not exercised that a certain amount of molasses, lubricating oils, leakages from pumps and pipes generally find their way into the drains and ultimately affect the character of the waste waters. The effects of all these wastes can be mitigated by providing suitable drains and better housekeeping in the factory.

An experimental pilot plant is being constructed in one of the sugar factories to study a method of treatment of these wastes. The design of the pilot plant is based on the information gathered during the survey and earlier work carried in this connection by the Kanpur Sugar Technology Institute.

DISTILLERY WASTES.

The nature and composition of the liquid wastes from distilleries and their suitablitiy for chemical and biological treatment was studied during this period.

Representative samples of the spent wash were collected from the factory near Calcutta and analysed. The results of the analysis of the waste samples are summarized in Table II.

TABLE I.

Survey and analysis of liquid wastes form sugar industry.

Name of factory	Crushing capacity (tons/day)	Crushing season (days/year)	Liquid wastes G.P.D.	Process used*	pH	Suspended solids (ppm)	Oxygen consumed from $KMnO_4$ (ppm)	Remarks
Sarya Sugar Factory, Sardar Nagar (U.P.)	2,200	130	1,200,000	D.S.	7·3	1,440	980	Used for irrigation. No problem.
Daurala Sugar Factory (U.P.)	1,450	150	100,000	D.S. & D.C.	6·7	300	840	Used for irrigation. No problem.
Nawabgunj Sugar Mills (U.P.)	1,300	110	50,000	D.S.	7·5	1,242	940	Drains to the fields. Nuisance.
South Bihar Sugar Mills, Bhita (Bihar)	1,200	120	400,000	D.S.	...	...	...	Drains to open land. Nuisance.
Mawana Sugar Factory (U.P.)	1,000	150	80,000	D.S.	7·1	1,200	860	Drains to a nearby canal. Leads to water pollution.
Basti Sugar Mills, Basti, (U.P.)	1,000	70	192,000	D.S.	7·2	700	940	Drains to the fields. Great nuisance.
Khaleelabad Sugar Factory (U.P.)	850	100	80,000	D.S.	7·2	600	1,400	Great nuisance.
Basti Sugar Mills, Waltergunj (U.P.)	830	100	178,000	D.S.	7·3	1,000	900	Drains to the fields for irrigation purpose. No problem.

*D. S.=Double sulphitation.
D. C.=Double carbonation.

TABLE II.

Analysis of the distillery wastes.

(All figures except pH are in parts per million.)

Item	Range
pH	4·7–5·0
Colour	10,000–18,000
Solids (*a*) Total	15,760–58,110
(*b*) Volatile	11,160–42,600
(*c*) Suspended	4,420–21,020
Total nitrogen	23,500–30,000
Phosphorus (P_2O_5)	2,000– 2,200
Potassium (K_2O)	35,000–37,500
Five day 20°C. B.O.D.	17,000–27,000
Oxygen consumed	7,900–10,000
Ether-soluble matter	2,000– 2,530
Copper reducing sugars	900– 1,000
Rate constant (K) value of B.O.D./day at 20°C. (mixed with 0·1 per cent Sewage)	0·04–0·05

The results show that the wastes are acidic in reaction and have a high content of organic matter which is mostly in the dissolved state. The low rate constant for B.O.D. would show that the wastes are not readily fermentable.

Laboratory scale experiments on different methods of treatment of distillery wastes have been continued.

Plain sedimentation does not remove any significant amount of the solids from the waste. Treatment with chemicals, such as, alum, iron salts, lime, chlorine and bleaching powder was next tried. Qualitative experiments showed that alum and iron salts were not effective. Treatment with lime ($CaOH_2$) gave better results. Therefore, quantitative experiments were carried out with lime. The results of lime treatment experiments are summarized in Table III:—

TABLE III.

Effect of treatment with lime on settling efficiency of distillery wastes.

(Temperature range—96°C. to 98°C., average of four obsrvations).

Item of analysis	Raw waste	AFTER TREATMENT WITH LIME IN (PPM.) AND SETTLING FOR ONE HOUR:			
		5,000	10,000	20,000	50,000
Suspended solids (ppm)	7,820	7,196	1,186	478	225
Percentage reduction	...	(8·1)	(82·9)	(90·6)	(95·5)
Five days 20°C. B.O.D. (ppm)	23,750	21,125	9,875	6,875	6,687
Percentage reduction	...	(11·6)	(57·7)	(70·7)	(71·4)
pH	4·3	9·2	11·1	11·9	11·9
Colour (ppm)	15,000	15,000	10,000	5,000	5,000
Percentage reduction	...	...	(33·3)	(66·6)	(66·6)

The data presented show that best results are obtained when the waste is treated with 20,000 ppm (two per cent) of lime. With this treatment about 90 per cent of suspended solids and about 70 per cent B+O+D+ are removed. The pH of the waste is, however, rendered alkaline (11·9). A large amount of solids settles at the bottom and these may be used for recovery of useful products. A fair amount of colouring matter is also removed.

The effluent after lime treatment and settling for one hour has still a large concentration of organic matter (five day 20°C. B.O.D. of the order of 7000 ppm). The pH of the effluent is also unfavourable for direct biological treatment. The effluent was, therefore, treated with CO_2 to control the pH and diluted with water in proportion of 1:4 and 1:8, respectively, and inoculated with one per cent sewage before putting on an experimental trickling filter. A 2″ diameter, glass-tube filled with stones 1/4″ to 1/2″ size to a depth of 3 ft. served as the experimental filter and it was dosed at a rate of 5 lb. of B.O.D. per cubic yard. Observations on the trickling filter are being continued. The data collected so far are not adequate to draw any conclusion, but there are indications that secondary treatment of the effluent in the trickling filter may bring about 50 to 60% reduction in organic load.

Work on pre-digestion (anærobic treatment) of distillery waste prior to chemical and biological treatments is also under progress.

Byproducts recovery.

Distillery wastes contain 0·5 to 2 per cent of solids. When the waste is treated with lime, most of it is coagulated and settles down as sludge. The sludge was collected and analysed with a view to determine the feasibility of recovering lime and other useful products. The results of analysis of this sludge are presented in Table IV:—

Table IV.

Analysis of sludge obtained after treatment with two per cent lime.
(Average of two observations).

Moisture, per cent	Silica SiO_2	Iron and aluminium	Percentage on air dry basis						
			Calcium CaO	Magnesium MgO	Potassium K_2O	Organic carbon	Total nitrogen	Free alkalinity	Loss on ignition
9·4	6·8	2·25	40·5	5·4	0·02	2·5	0·26	124	35·5

The results indicate that it may be possible to recover a good proportion of lime from the sludge and thereby bring down the chemical cost for treatment.

Further work on recovery of other byproducts from the sludge is in progress.

INDUSTRIAL WASTE DISPOSAL AND WATER POLLUTION RESEARCH UNIT.
ALL-INDIA INSTITUTE OF HYGIENE & PUBLIC HEALTH, CALCUTTA.

Industrial waste survey—sugar mills.

1. Plant

State District Town or village Railway station

2. Location

3. Season when worked

4. Information: (*a*) Name

(*b*) Address

5. Plant operation: Hours per day Days in week Number of days in year

6. Water consumption: Gallons per day average Treatment

Drinking ....

Industrial ....

Cooling ....

7. Raw materials: Cane tons per day per year

8. Chemicals used in manufacture:

Lime ....

Sulphur ....

Others ....

9. Process used: C DC S DS

10. Products obtained

11. Wastes: Quantity Character Analysis

Solid

Liquid:

(*i*) Condenser water

(*ii*) Milling house washing

(*iii*) Centrifugal

(*iv*) Boiler house

12. Present method of disposal and difficulties

13. Possible spills

14. Sanitary sewage disposal:

    Persons

    How disposed of

    Whether this gets mixed up with waste

15. Other information:

    Gaging facilities

    Future programme

16. Survey by: Date

4. Study of the septic tank latrine for the safe disposal of human excreta under Mr. F. K. Erickson at the All-India Institute of Hygiene & Public Health, Calcutta.

Object:—The National Water Supply and Sanitation programme has given considerable emphasis to the improvement of rural and semi-rural sanitation measures. Although the dug-well latrine and bore-hole latrine suit a great proportion of the rural areas, there are many sections where these are not suited due to soil conditions, ground water table or compactness of village housing. In some of these instances the 'septic tank latrine' has been suggested as a workable solution. Due to the relative cost of the standard septic tank, few installations in a strictly rural single family arrangement are known. To effect the most of economy in construction, due advantage needs be taken of factors such as minimum required capacity and simple measures for effluent disposal. To arrive at these economics, more data are needed on the quantity of liquids and solids to be anticipated per capita, the relationship of these factors to the necessary capacity and the character of the effluent in terms of its safe disposal.

Synopsis of the work done:—During the six months of the year 1955-56 that the project has been started, much time has been consumed in recruitment of the personnel. The Research Assistant was finally selected in August and has been oriented to the testing procedures to be used. Facilities have been provided for him to do the analyses at the Public Health Laboratory at the Rural Health Centre, Singur, and equipments and supplies have been provided or are under procurement.

Sites for two septic tank latrines have been obtained on agreement with several families of the Singur area. Because of need to include facilities for sampling of both the normal influent to the tank as well as the effluent, locations which permitted appreciable elevation differences had to be secured. Designs for two tanks have been prepared. One is a standard unit for ten persons and is being constructed for a single family of nine persons. The other is for a joint family of fifteen persons for which a standard design for twenty users has been selected with provision for modifying the depth to reduce capacity after an initial six months operation at this 'extra' capacity. Designs have been modified to permit variation of inlet and outlet baffles also. Actual construction of these units is underway although they have been delayed by the required procedure for cement permit and the monsoon conditions of the area. Work is being carried out by direct employment of labour under the supervision of Mr. A. V. Rao, Assistant Engineer (Public Health), Rural Health Unit & Training Centre, Singur.

The following schedule of testing has been established:—

1. Influent to tank—24-hour composite—weekly for first 3 months; fortnightly thereafter.

   Determining—

   (*a*) Total volume/24 hours.
   (*b*) Total solids-organic and inorganic.
   (*c*) Settleable solids-organic and inorganic.
   (*d*) B.O.D.
   (*e*) Oxygen absorbed—4 hours 25°C.
   (*f*) Microscopic-ova count.

2. Effluent from tank "grab" samples—taken three times weekly at regular intervals.

   Determining—

   (*a*) Total solids-organic and inorganic.
   (*b*) Settleable solids-organic and inorganic.
   (*c*) B.O.D.
   (*d*) Oxygen absorbed—4 hours 25°C.
   (*e*) Microscopic-ova count (once a week only).

PHARMACOLOGY.

1. Indigenous drugs inquiry under Dr. H. Hausler and Dr. M. L. Chatterjee at the School of Tropical Medicine, Calcutta.

Interaction of Rauwolfia serpentina *alkaloids* (*R.S.*) *with neurotransmitter agents.*—The rôle of acetylcholine in regulating and controlling the functions of the brain, heart and other automatic organs has been repeatedly stressed by various workers. The systemic blood pressure (which *Rauwolfia* alkaloids depress) is on the whole dependent on an interplay of these functions.

Opinion is divided regarding the mechanism of action of *Rauwolfia* alkaloids (R.S.) in relation to their power of modifying or controlling the action of the local hormones, namely acetylcholine, adrenaline and histamine, which incidentally, have important functions regulating the physiological activity of heart, of blood vessels and capillaries, as also of muscles, voluntary and involuntary. All these three substances have been found in brain and C.N.S. although their rôle in the function of the latter is not yet understood. Of these, acetylcholine only, according to the generally accepted view, has been assigned a very important hormonic and regulating influence on those functions on which not only depend to considerable degree, the systemic B.P. but as well the condition of sleep. The effect of adrenergic stimulation is also a function of acetylcholine liberated at the end of the preganglionic autonomic nerves. It is only natural and of interest to take up investigation into the effect of R.S. on the action of acetylcholine following the discovery of the unique central action and the peripheral adrenolytic and antiadrenergic action of R.S. Since the site and mechanism of drug action and the phenomenon of antagonism are best understood and interpreted from experiments with isolated tissues, a series of experiments have been done on the phramacology of the alkaloids of *Rauwolfia serpentina* Benth (R.S.) on various isolated tissues.

A. ANTAGONISM AGAINST THE SPASMOGENIC EFFECT OF ACETYLCHOLINE.

(1) *On plain muscles.*—In 12 experiments using guinea-pig ileum, it was found that an extract containing total soluble alkaloids in itself would cause a variable, transient and feeble spasmogenic action especially after a high concentration, e.g., 5×10^{-5} g./c.c. in the final solution; further, when a suitable spasmogenic dose of acetylcholine (e.g., 10^{-8} g./c.c. in final solution) was applied 30 to 50 seconds after the administration of R.S., there was a competetive inhibition of the action of acetylcholine depending on the dose of R.S. used. A concentration above 5×10^{-5} g./c.c. of alkaloids completely antagonized the action of acetylcholine.

(2) *On cardiac tissue.*—In experiments on isolated auricles of rabbit-heart suspended in a suitable physiological medium it was observed that following the application of a dose of R.S. (e.g. 5×10^{-5} g./c.c. in the final solution) before and after atropine (which was sufficient to block the action of 10 times the dose of acetylcholine used as control), the degree of depression of rate and amplitude following R.S. did not vary significantly, e.g., the depression of auricular amplitude before and after atropine was by 31·08 and 29·4 per cent, respectively, and that of rate of auricular beat was by 20 and 17·4 per cent, respectively.

The action of acetylcholine, on the other hand, was greatly reduced or abolished after a dose of R.S. This reduction was more marked after changing the *Rauwolfia* solution for Ringer-Locke's. A long time, sometimes two hours, was required in order to regaining the control effect.

(3) *On striated muscles: Rectus abdominis muscle of Toad* (Bufo-melanostictus): *Antagonism of R.S. against the nicotinic action of acetylcholine.*—Studies of the action of R.S. alkaloids on striated muscles of food have apparently not been attempted before. The action of suitable doses of acetylcholine (Ach) (5×10^{-8} to 2×10^{-7} g./c.c.) as control was measured and compared with identical doses of Ach in the presence of a known concentration of R.S. (varying from 2×10^{-7} to 10^{-4} g./c.c.) applied three minutes earlier than acetylcholine. Concentration of R.S. higher than 2×10^{-7} g./c.c. tended to increase the tone of the muscle and raised the level of the base line; concentration of R.S. from 10^{-5} g./c.c. and higher would produce marked increase in tone in course of three minutes and even throw the muscles into a variable degree of contraction depending on the duration of contact with R.S. The application of a subsequent dose of acetylcholine lessened the degree of contraction and the antagonism was of a competitive nature. Again, the degree of reduction of the action of Ach by R.S. and also the time required for the muscle to recover fully were found to depend on the concentration of R.S. and the duration of its contact with the muscle. Overnight old or eserinized rectus was affected similarly by R.S. but the susceptibility to Ach seemed to recover slightly earlier. Complete blocking of the action of acetylcholine was, however, not observed even in experiments where acetylcholine was tested in solutions of frog Ringer containing R.S., on muscle strips previously kept in contact for 12 hours or more with varying concentration of R.S. These observations indicate that the antagonism of R.S. is of a competitive nature and further, that R.S. apparently does not modify the enzyme cholinesterase of the tissue.

B. ANTAGONISM OF R.S. AGAINST THE ACTION OF ADRENALINE.

(1) *On the isolated auricles of rabbit.*—In contrast to earlier work on the action of adrenaline on the auricles and ventricles of heart *in situ*, isolated auricles have been utilized here to study this antagonism. In altogether 10 experiments the action of suitable dose of adrenaline before and after varying doses of R.S. were compared. In some experiments the effects of doses of R.S. increasing in geometric progression were used for a fixed period before adrenaline was applied. It was noted that excepting in the presence of the highest concentration of R.S. used, viz. 2×10^{-4}g./c.c., the percentage of increase of rate caused by adrenaline was not greatly altered even in presence of R.S. in the bath. The percentage of increase of the amplitude of beat, however, was modified by R.S. Lower concentration, such as 5×10^{-6} g./c.c. of R.S., caused occasionally a slight augmentation of the height of the normal amplitude and of the action of a subsequent dose of adrenaline, especially in an auricle exhausted through handling. The usual effect of R.S. on the action of adrenaline,was to cause progressively a greater diminution of the percentage of increase of amplitude caused by a particular dose of adrenaline as control.

(2) *On the isolated duodenum of rabbit.*—The property of adrenaline to relax the duodenum of rabbit was utilized for studying the antagonism of R.S. There appears a less degree of relaxation after adrenaline in

presence of R.S. in the bath compared to that caused by adrenaline alone (control), especially when R.S. is left in contact for sometime. The character of response was found to be altered in the latter condition and also after a heavy dose of R.S.

There are divergent views regarding the effect of R.S. on the action of adrenaline in intact animals; the above experiments indicate that the said effect may be different depending on the dose and the concentration of R.S. used before the administration of adrenaline.

C. ANTAGONISM OF R.S. AGAINST THE ACTION OF HISTAMINE.

This was also studied on guinea-pig ileum (isolated). The drug R.S. reduced or completely neutralized the effect of a control dose of histamine (viz., 2×10^{-8} g./c.c. in final solution). There was a progressive reduction of the action of histamine following an increasing concentration of R.S. in the final solution.

Incidentally, it may be mentioned that the total alkaloids of R.S. in two per cent HCl extract, was found to reduce also the action of barium chloride on guinea-pigs ileum.

Chemical investigations on Rauwolfia *alkaloids employing the technique of Countercurrent Distribution.*—With the help of an 80 tube Countercurrent Distribution Apparatus designed and constructed under direct supervision of the inquiry, it has been possible to fractionate the crude alkaloids of *Rauwolfia serpentina* into various fractions consisting of pure alkaloids. Besides making possible the characterization of the various alkaloids in respect of their partition co-efficient between chosen solvent pairs (data in themselves of importance in isolation and purification procedure) the successful development of the technique has made it possible to undertake investigation on the pharmacology of some of the individual alkaloids not yet studied. Findings include the following:—

(1) Total soluble alkaloids of *Rauwolfia serpentina* Benth (Dehra Dun) in chloroform solution resolve into more than eight pure alkaloidal fractions following countercurrent distribution between chloroform and 0·2. M phosphate buffers (having pH 4·7., 5·6., 8·6. and 1·8, respectively). In Dehra Dun variety the above eight alkaloids having appreciable partition co-efficient in buffer/chloroform phase constitute more than 85 per cent of the total alkaloids. Other fractions occuring in minute quantities as well as those in the 'oleoresin fraction' are being investigated.

(2) Investigations on the highly fluorescent character of a solution of total alkaloids from R.S. (Dehra Dun) *vis-a-vis* its prospective application for the purpose of estimation of micro-quantities of alkaloids in biological materials has revealed that the total fluorescence is associated with very little mass and mostly do not pertain to any principal alkaloid. An alkaloid fraction possessing hypotensive activity and at the same time having a strong associated fluorescence has been isolated and awaits identification. Preliminary *in vitro* experiments show good recovery of the fluorescence from biological fluids and tissues and point to the alkaloid as being suitable for metabolic studies. Thus, the possibility of a new field of research on R.S. is being envisaged.

2. Indigenous drugs inquiry under Bt.-Colonel R. N. Chopra at the Drug Research Laboratory, Jammu.

This small pharmacological unit has been set up by the Indian Council of Medical Research to carry out systematic screening studies of indigenous drugs derived from the rich flora of the North-Western Himalayan region. The staff consists of one Assistant Pharmacologist, one technician and one peon. The work is being done in close collaboration with the Pharmacologist, Research Chemist and Botanist of the Drug Research Laboratory. The authentic specimens of drugs are collected by the Botanist in the field and are then finally identified in the laboratory before the Chemists take it up for analysis and for preparation of solutions for testing.

A number of medicinal plants commonly used by the practitioners of Indian medicine have already been screened and results published. Besides, indigenous substitutes for a number of pharmacopœial drugs which do not grow in the country, have been worked out from the allied species growing in the country. Some of these, e.g., *Strophanthus* and *Dryopteris* species growing in India, have already been investigated and shown to be good substitutes for the official drugs. This work is of economic importance and is being continued.

Detailed pharmacological studies of a number of essential oil-bearing plants which grow in this region and are commonly used by indigenous practitioners or are of economic importance have been carried out and results published. About half a dozen of these essential oils have good prospect of being utilized in the soap, cosmetic and perfume industries in India. Others, such as *Nordostachys jatamansi*, are of great medicinal interest on account of their sedative properties on central nervous system.

Antibiotic principles occurring in locally grown medicinal plants, such as *Azardirachta indica*, *Leea hirta*, *Cassia absus*, *Adhatoda vasica* have been studied and results published. Work on anti-tubercular principles occurring in indigenous plants, such as *Allium schœnoprasum* and *Alpinia galanga* is in hand. Along with this, studies on the antibacterial properties of a number of indigenous medicinal plants used in Ayurvedic and Unani medicine in the treatment of various infective conditions, have been started. About 20 plants have been screened so far.

Work on preliminary testing of reputed abortificient plants was continued and 24 such plants have been investigated. *Randia dumetorum* (Arar), *Momordica charantia* (Karela), *Sapindus trifoliatus* (Retha), *Cuscuta reflexa* (Akashbel), *Cuscumis trigonous* (Indrayan), *Lepidium sativum* (Halim) and *Trianthema mongyna* (Itsit) have been found to stimulate the uterine contractions *in vitro*.

The importance of these screening studies is that these have led the way for the detailed studies to be taken up by workers with larger laboratory facilities and to the discovery of plants, such as *Rauwolfia serpentina* and others now well-known remedies in the treatment of diseases.

This inquiry has also been busily engaged with the preparation and publication of a *Review of work on Indian Medical Plants* including indigenous drugs. The preparation of index of this book of about 300 pages has in itself been a very laborious undertaking. This work has, however, been completed and the book will be ready for presentation in its final form.

This small Unit has been the training ground of a number of young pharmacologists and technicians of whom there is a great shortage in the country. The trainees have gone out and are working in various pharmacological laboratories in different parts of India. The Pharmacology Committee have noted this and have recommended that I.C.M.R. Research Scholars may be sent to this Unit for training.

In the Second Five Year Plan proposals have been put up for the establishment of a Medical College in Kashmir State. This Unit will be able to serve as a nucleus for the starting of the department of pharmacology of this institution.

DETAILS OF WORK DONE.

I. INDIGENOUS DRUGS.

Our studies on the screening of indigenous drugs were continued. Preliminary studies with *Nyctanthes arbortritis* Linn. and *Celastrus panniculatus* Willd. showed that the active principles contained produced relaxation of the smooth muscle of the intestines and uterus as well as of the musculature of blood vessels. The other two drugs screened, i.e., *Didymocarpus pedicellata* and *Cedrella toona* Roxb. showed no activity whatever. The details of experimental work are given below:—

1. *Nyctanthes arbortistis* Linn. Oleaceæ family (Vern.-H.- Siharu).— This is a large shrub or a small tree, rough all over with stiff whitish hairs growing in the outer Himalayan ranges from Kashmir to Nepal, Assam, Burma, Bengal and Central India. It is also cultivated in many parts of India. The plant has been in extensive use both in the Ayurvedic and Tibbi medicine. The juice of leaves is bitter, acrid and is believed to cure fever, rheumatism and sciatica. The bark is used in the treatment of bronchitis. The flowers have a bitter unpleasant taste and are said to have stomachic and carminative properties; they have an astringent action on the bowels. The plant is also reported to possess antibilious, expectorant, cholagogue and laxative properties. The leaves contain an alkaloidal principle and an essential oil. A glucosidal principle has also been discovered in the flowers and a bitter principle called nyctanthin has also been found.

For preliminary pharmacological studies an alcoholic extract of the leaves, which contain all the active principles, was used and the results noted below:—

Carotid blood pressure.—In dogs anæsthetized with phenobarbitone (100 mg./kg.) the graded doses of the extract from 10 mg./kg. to 20 mg./kg. were given intravenously. A sudden fall in blood pressure was produced followed by a rise to normal level within a short time. Amplitude of heart beats increased and frequency decreased. The fall of blood pressure is very little affected by previous cuttings of the vagi and atropinization.

Blood vessels.—With the fall ,in blood pressure the intestinal volume decreased but returned to normal with the rise of blood pressure. Spleen volume is also decreased with doses of 15 and 20 mg./kg. showing that blood vessels of the whole of the splanchnic area are constricted.

Perfusion of blood vessels in hind leg of the dog with the extract showed that outflow of perfused fluid is increased after administration of the extract in a dose of 20 mg. The outflow does not return to normal for a long time, showing a marked dilatation of blood vessels.

Heart in situ.—The extract in a dose of 20 mg./kg. is found to decrease the amplitude of the contractions of both the auricles and the ventricles of the heart in dogs. The rate of contractions is also decreased. Continuous infusion of the extract in guinea-pigs causes gradual decrease in frequency and amplitude of cardiac contractions, till the heart stops in diastole, probably due to direct effect on the cardiac muscle.

Isolated heart.—Perfused by Langendorf's method, the isolated rabbit heart showed depression with doses from 5 mg. to 7 mg. added to the perfused fluid. The heart returns to normal after some time. Larger doses stop the heart altogether within a few minutes. The effect is probably on the heart muscle.

Respiratory system.—In dogs anæsthetized with phenobarbitone doses of 10 mg./kg. to 20 mg./kg. of the extract produced transitory stimulation of the respiratory movements, both the amplitude and the respiratory rate being increased. The respiration, however, returns to normal after a short time. No change in respiration was observed after atropinization and after section of the vagi. The effect probably is not central.

Intestines in situ.—The extract produced immediate relaxation of the longitudinal musculature of the small intestine *in situ* as recorded by Jackson's Enterograph with doses of 10 mg./kg. to 20 mg./kg. given intravenously in anæsthetized dogs. The movements return to normal preceded by slight stimulation.

Isolated intestine.—Isolated rabbit's small intestine in Dale's bath shows instantaneous decrease of tonus and amplitude of its rythmic contractions and later on complete inhibition, in a dilution of 1 in 2,000. With higher concentration relxation was more pronounced. The contractions produced by barium chloride and acetylcholine chloride were antagonized in a dilution of 1 in 1,000. After atropinization the contractions produced by barium chloride are also inhibited.

Uterus in situ.—The extract given intravenously relaxed the contractions of uterus of anæsthetized dogs in doses of 10 mg./kg. and above.

Isolated uterus.—Isolated cornu of guinea-pig uterus Dale's bath showed relaxation of its normal rythmic movements in a dilution of 1 in 250. The rat's uterus is also relaxed in dilutions of 1 in 1,000.

Conclusions.—From the experimental data given above it may be concluded that the effects of the extract are not very marked on cardiovascular and respiratory systems and, therefore, the drug is probably not important from the point of view of these systems. The action on the plain muscles of the intestines and the uterus, however, is well marked as these are relaxed even after induced myogenic and neurogenic spasms. The antispasmodic action appears to be direct on musculature and the drug may be useful in lowering blood pressure.

For further studies the active principles of the plant, i.e., the alkaloid and the glucoside and nycatanthine are being isolated and detailed pharmacological studies will be carried out.

2. Celastrus panniculatus *Willd.* (vern.-Malkangni):—This is a large deciduous climber commonly found in sub-Himalayan tracts from the Jhelum eastwards up to an altitude of 6,000 ft. above sea-level. It is

also grown throughout the hilly parts of Bombay, Gujrat, Central India and Madras. The leaves are used as emmenagogue. In Ayurvedic and Unani systems of medicine, the seeds are said to be hot, bitter, laxative, aphrodisiac and powerful nervine tonic. The fixed oil from the seeds is commonly used by Hakims and Vaids as stomachic, and tonic, to cure cough, asthma, leprosy, rheumatism and paralysis. The plant contains alkaloids celastrine, paniculatine, a glucoside, some colouring matter and fixed oil.

The preliminary pharmacological studies of the fixed oil from the seeds and their alcoholic extract which contains the alkaloids and glucoside were carried out.

(*a*) *Systematic blood pressure.*—The action of alcoholic extract from seeds was studied on the carotid blood pressure of dogs, anæsthetized with phenobarbitone. Alcoholic extract in doses of 10 to 30 mg./kg. produced fall in blood pressure followed by subsequent gradual rise. Larger doses lower the blood pressure permanently. Heart rate is decreased and amplitude increased. The fall in blood pressure is also observed after the termination of the vagi have been paralysed with atropine, showing that vagal inhibition does not play much part in the fall which is produced.

As the fixed oil is also used by practitioners of indigenous medicine, small dose given intravenously produced similar effects to the extracts though not to such a marked degree.

Heart in situ.—Myocardiographic experiments in anæsthetized dogs showed diminution in the amplitude of the auricular and ventricular contractions with doses of 5 mg./kg. of the extract. This effect is not abolished if the vagi are cut in the neck or their terminations paralysed with atropine. It would appear, therefore, that the effect is direct on the cardiac muscle.

Isolated heart.—Isolated hearts of rabbit were perfused according to Langendorf's method with oxygenated Locke's solution. Addition of five mg. of the extract produced depression of the heart, both the amplitude of the contractions and frequency of the beats being decreased. With higher doses the depression was immediate and well marked. Minute doses of the fixed oil also produced similar effect.

Intestinal and spleen volume.—The effect on the blood vessels of the splanchnic area was studied by noting the changes produced on the volume of the spleen and the intestines. With the fall in blood pressure both showed a decrease in volume.

Respiratory system.—Graded doses of the extract given intravenously to anæsthetized dogs produced stimulation of respiratory movements. The amplitude of respiratory movements was increased and the frequency of respiration was also stimulated. This phenomenon was still observed after the paralysis of vagal nerve-endings with atropine.

Alimentary system.—The effect of the alcoholic extract in doses of 10 mg. to 20 mg. on the intestines *in situ* was studied by Jackson's enterograph on dogs under phenobarbitone anæsthesea. After the drug was given into the femoral vein there was a distinct inhibition of both the tonus and the peristaltic movements of the intestines. With larger doses complete inhibition of the movements occurred. The contractions produced by barium chloride and acetylcholine chloride were also antagonized. Small doses of the oil given intravenously produced similar effects, showing that similar active principles were present in the oil.

Isolated intestine.—The effect of both the fixed oil and alcoholic extract on isolated pieces of rabbit's intestine was studied in Dale's bath oxygenated Tyrode's solution. In dilutions of one in 500, the oil relaxed the tone of the musculature as well as the pendulum movements. The extract produced relaxations in dilutions of one in 2,000. The contractions produced by barium chloride and acetylcholine chloride were also antagonized.

Isolated uterus.—Isolated uterus of guinea-pigs in Dale's bath showed relaxation in dilutions varying from one in 1,000 to one in 500 of the extract.

Conclusion.—From the experimental data gathered, it may be concluded that the alcoholic extract from this plant produced a well-marked depressant action on the cardiovascular system and a slight stimulating action on the respiratory movements. A well-marked relaxation of the smooth muscle of the intestine and uterus is also produced. The fixed oil also produced a similar effect though in a much milder form, showing that probably some of the alkaloidal or glucosidal principles present have passed into the oil during expression.

Further work on the isolation of active principles in pure form will be taken up.

3. Didymocarpus pedicellate R.Br.—The plant grows in sub-tropical regions from Chamba to Kumaon at altitudes from 2,500 ft. to 5,000, ft. above sea-level. The plant contains pedicin one per cent, isopedicin 0·4 per cent, pedicinin 0·3 per cent and pedicellin one per cent. All these are crystalline substances. The leaves are used by the indigenous practitioners for the treatment of stones in kidneys and bladder. The pharmacological action of the alcoholic extract of the leaves was studied but did not show any marked pharmacological activity.

Conclusion.—From the experimental data obtained it may be concluded that the drug is not likely to be physiologically very active and further studies may not lead to useful results.

4. *Cedrela toona Roxb.*(Vern. Tuni).—This is tall tree, having numerous branches and forming a shady head and found in sub-Himalayan tract from the Indus eastwards to Chittagong, Assam, Burma, Western Ghats and other hills of western peninsula. The bark has a sharp acrid, sweetish taste and is used by Ayurvedic practitioners to cure fevers, headache, biliousness and Unani practitioners use the bark as a bitter, cardio-tonic, aphrodisaic and expectorant. The alcoholic extract of the bark has been investigated by passing through various tests but did not show any pharmacological activities.

Conclusion.—From the experimental data obtained it may be concluded that this plant does not show any marked physiological activity and that further studies may not be profitable.

II. ABORTIFACIENT PLANTS.

The study of pharmacological properties of the indigenous plants used in the treatment of uterine disorders by the indigenous practitioners, was started last year and action of *Randia dumetorum* (Arar), *Sapindus trifoliatus* (Ritha), *Cuscuta reflexa* (Akashbel) *Apium graveolens* (Ajmod), *Abrus precatorius* (Rati), *Daucus carota* (Gajar), *Gossypium herbaceum* (Kapas), *Carica papaya* (Papita) and *Withania somnifera* (Asgand) on the isolated guinea-pig's uterus was reported. When this preliminary testing of the whole of this group is completed further advanced experimental work will be taken up in case of those showing marked physiological activity. This preliminary work was continued during the present year and following new plants were tested according to the method stated above:—

Serial number		*Vernacular*	*Part used*
1.	*Memordica charantia*	Karela	Roots
2.	*Cucumis trigonus*	Indrayan	Seeds
3.	*Plumeria acutifolia*	Gobur champa	Bark
4.	*Trianthema monogyna*	Itsit	Whole plant
5.	*Lepidium sativum*	Halim	Seeds
6.	*Achyranthes aspera*	Apamarga	Whole plant
7.	*Semicarpus anacardium*	Bhilawa	Seeds
8.	*Trianthema decandra*	Itsit	Whole plant
9.	*Crotolaria juncea*	...	Leaves
10.	*Celastrus panniculatus*	Malkangni	Seeds and leaves
11.	*Taxus baccata*	Birmi	Leaves
12.	*Aristolochia bracteata*	Kiramar	Whole plant
13.	*Artemisia vulgaris*	Nagodana	Whole plant

Conclusion.—Out of 13 plants above noted, the first six, namely, *Momordica charantia*, *Cucumis trigonus*, *Plumeria acutifolia*, *Trianthema monogyna* *Lepidium sativum*, *Achyranthes aspera* produced a definite stimulant action on the guinea-pig uterus, increasing the height and frequency of contractions. The action was well marked in higher concentrations. *Trianthema decandra* and *Semicarpus anacardium* have a weak action on the uterus, as these stimulate the contractions in dilutions of one in 250 only. The remaining drugs did not show any stimulation. The extract of *Momordica charantia* stimulates the uterine movements up to dilutions of one in 50,000, *Cucumis trigonus* seeds one in 5,000, *Plumeria acutifolia* one in 2,000. *Trianthema monogyna* one in 1,000 and *Lepidium sativum* one in 1,000. *Atropinization* did not relax the tonic contractions so that the effect was probably on the musculature.

Comparative study.—In this study experiments were performed on isolated guinea-pig uterus to evaluate comparative estimation of the oxytocic activity of these drugs which were found to have stimulant action on the uterus of guinea-pigs.

Method.—The method adopted was the one described by Burn and Dale (1922) for the standardization of the oxytocic principle of the pituitary extract. The comparison was made by finding out a suitable dose of the drug, which would produce contraction of a guinea-pig uterus nearly equal to that produced by a sub-maximal dose of the standard pituitary extract. The rise in height produced was considered as the criterion for measuring the activity of the drug. The maximum rise produced was measured and by applying simple mathematical calculation a dose of the test drug which would produce the same rise, as would be produced by I.O.I.U. (Oxytocic unit) of the standard was estimated. These experiments showed that *Cucumis trigonus* was the most potent in its oxytocic activity as compared with the standard. Next in descending order came *Momordica charantia*, *Randia dumetorium* and *Lepidium sativum*. Further investigations are in progress and will be reported in due course.

III. Screening of antibacterial properties of indigenous drugs.

In Ayurvedic and Unani systems of medicine a large number of indigenous plants have been used by Vaids and Hakims for the treatment of infective diseases. In recent years some workers in India have succeeded in isolating antibiotic substances from medicinal plants. Pristimerin (isolated from *Pristimera indica*) and Ptergospermin (isolated from *Moringa Ptergosperma*) show that higher plants do seem to offer great chance of containing substances possessing marked antibacterial properties.

A number of indigenous plants alleged to possess antibacterial properties have been selected for detailed investigations. As a preliminary step, the alcoholic extracts of the parts noted against each of the following ten plants, were tested for their antibacterial activity by the serial dilution method against *M. Pyogenes var Aureus*, *Streptococcus pyogenes*, *Shigella shigæ Salmoneալla typhosus*, *Escherichia coli*, *Klebsiella penumoniæ* and *B. Proteus vulgaris.*

Serial number	*Plant*	*Parts examined*	*Effect at concentration of 1mg./c.c.*
1.	*Abrus precatorius* (ver. Ratti) ...	Leaves roots, seed & stems	+
2.	*Abutilin indicum* (ver. Kanghani)	Leaves & seeds	+
3.	*Corydalis govoniana cern Bhutkesi*)	Tubers	+
4.	*Terminalia chebula* (ver.-Harrar) ...	Fruit	+
5.	*Cassia angustifolia* (ver. Sana) ...	Seeds	+
6.	*Butea monosperma* (ver. Plash) ...	Seeds, leaves	+
7.	*Psidium guyava* (ver. Anjirzard) ...	Fruit	+
8.	*Cuminum cyminum* (ver. Zira) ...	Seeds	+
9.	*Lepidium sativum* (ver. Zira) ...	Seeds	+
10.	*Apium graveolens* (ver. Ajmod) ...	Seeds	+

Note.— + indicates full growth. Broth at pH 7·2 was inoculated with 5 mm. loopful of 24 hours broth cultures and the results read after 24 hours of incubation at 37°C. The extracts in other solvents against the above-mentioned organisms and the antitubercular properties are under investigation.

3. Investigation into the study of adrenal cortical hormones under Dr. B. Mukerji at the Central Drug Research Institute, Lucknow.

During the year under review, the problem was approached from two angles:—

(A) The inter-relationship of male sex hormone (testosterone) with thyroid and adrenocortical hormone.

(B) The effect of hyperthyroid status on the eosinopenic influence of cortisone.

(A) TESTOSTERONE AND ADRENOCORTICAL ACTIVITY.

It is believed that testosterone may be responsible for adrenal atrophy in intact animals, but may prevent adrenal atrophy in corticone-treated animals. And yet another group of workers found no change in the size of the adrenal of rats administered testosterone. To clarify the matter, the following work was undertaken:—

(*i*) *Effect of testosterone propionate on the adreno-cortical activity of young male rats.*—Male albino rats, weighing 41·4±4·7g., from the Institute colony were employed in this study. Groups of twelve rats each were divided into two groups of six each. One group was treated with testosterone propionate (6 mg., dissolved in 0·25 c.c. of sesame oil, per rat on alternate days) and the other group served as a control, receiving the same quantity of oil alone. The treatment period was altogether 16 days and the animals were sacrificed on the day following the final injection. The adrenals were carefully dissected out, weighed and studied histologically. Since thyroid bears a close relationship with adrenal gland (*vide infra*), a study of the former tissue was also undertaken side by side. Typical results are given in Table I:—

TABLE I.

Adrenal weight of normal and experimental rats.

Group number	Treatment	Average initial body-weight, g.	Average final body-weight, g.	Average body-weight gain, g.	Mean adrenal weight (mg.) with S.E.
I.	Normal control	42·3	59·8	17·5	14·1±·36
II.	Testosterone-treated ...	40·5	59·2	18·7	21·7±·77

The mean weight of the adrenal glands are presented in Table I along with the body-weight of the respective groups of rats. It is evident that the weight of the adrenals of the testosterone propionate-treated animals are heavier than those of the control animals (statistically significant $P < \cdot 001$). This change in the adrenal weights bears no relation with the body-weight changes in the animals.

Histological examination of the adrenals in the adrogen-treated group also reveals considerable stimulation of the gland showing hypertrophy and hyperplasia of the cells. The thyroid gland of the androgen-treated animals reveals marked hypertrophy and hpyerplasia of the acinar cells with lessening of the acinar colloids, which signify that the gland is in an hyperactive stage.

Conclusion.—Injection of testosterone propionate, under the experimental conditions mentioned, exerts a stimulating influence on the adrenal cortex of young male rats. The concomitant changes in the thyroid is a significant pointer in this respect. It is presumed that the stimulating effect of the testoid on the adrenal cortex may result from increased ACTH output brought about by hyperactive thyroid, following androgen administration.

(*ii*) *Effect of testosterone propionate on the adrenal cortex of experimental hypothyroid rats.*—Male albino rats, weighing 52·5±6·7 g., from the Institute colony were employed in this study. Twenty-four rats were divided into three groups of eight each. Sixteen rats were treated with subcutaneous injections of ‘Thiourea’, the dose of which was gradually increased from 4 mg. to 20 mg. per rat daily. Total period of treatment was 88 days. A group of eight rats from these thiourea-treated animals received intramuscular injections of testosterone propionate (8 mg., dissolved in 0·25 c.c. of sesame oil, per rat on alternate days) from the 65th day of thiourea treatment. Androgen treatment period was spread over 23 days. Rest of the animals served as control, receiving the corresponding quantity of the diluents.

The animals were sacrificed on the day following final treatment. The adrenals were carefully dissected out and weighed. As a measure of the secretory activity of the gland, ascorbic-acid content was estimated by the method of Bessey *et al.* (1947). The total cholesterol content of the adrenal was determined according to the method of Roy *et al.* (1955). The results are indicated in Table II:—

TABLE II.

Adrenal weight and its ascorbic acid and total cholesterol content.

Group number	Treatment	Mean adrenal weight (mg.) with S.E.	Mean ascorbic-acid content (mg./100 g. of adrenal tissue) with S.E.	Mean cholesterol content (mg./g. of adrenal tissue) with S.E.
I.	Normal control	22·7±1·06	475·0±16·3	42·3±1·97
II.	Thiourea ...	17·5±0·75	284·2±17·3	34·5±0·7
III.	Thiourea *plus* testosterone propionate ...	23·4±1·21	274·8±16·6	22·1±1·92

It is noted that the adrenals of thiourea-treated animals are much lighter than those of control animals. Testosterone therapy reverses the change and brings back the weight of the gland to its normal value.

The diminution in the content of cholesterol and ascorbic acid in the adrenal of hypothyroid rats is not a contradictory finding, but taken along with the atrophy of the adrenal tissue reveals that due to diminished activity of the gland for sometime, the steroid output has diminished along with less elaboration of precursor substances. Further diminution of adrenal constituents following testosterone administration is probably due to increased activity of the gland—an explanation which seems to be in conformity with the increased size of the gland.

Conclusion.—Hypothyroidism due to prolonged administration of thiourea leads to adrenal atrophy with the resultant lowering of the secretory activity of the cortex. Treatment with testosterone-propionate corrects the atrophic change and improves the secretory status of the gland in such animals.

The present result shows that testoid therapy also improves the adrenal function in rats whose thyroid is geared at a lower level of activity. Further work is in progress to elucidate the precise rôle, the thyroid might play in such testoid action.

(B) THYROID STATUS AND EOSINOPENIC ACTIVITY OF ADRENOCORTICAL HORMONE.

Following standardization and comparative study of the bioassay methods for oxycorticoids (*vide* Indian Council of Medical Research Annual Report, 1954), the effects of hyper and hypothyroid conditions on the eosinopenic property of cortisone are being studied, to see whether thyroid status has got any bearing on the bioassay procedures employed.

Studies on the effect of hyperthyroid condition on the eosinopenic influence of cortisone.—Male albino rats, weighing about 120 g., were employed in this study. Animals were adrenalectomized and saline was given in place of drinking water. The operated animals were treated with thyroxine at a dose of 30 μg. per rat daily for three days prior to the day of experiment. On the morning of the day of experiment, the animals were given 40 μg. of thyroxine followed by 100 μg. of adrenaline. Blood was collected after four hours for the initial eosinophil count. The rats were immediately injected with cortisone and blood was subsequently collected at three, six and ten hours intervals.

In the control animals, same procedures were followed except that equal quantity of distilled water was administered in place of thyroxine. Total eosinophil counts and other procedures of the experiment were the same as followed by Roy *et al.* (1955). Experiments were undertaken at different dose levels of cortisone.

Conclusion.—Preliminary results tend to show that thyroxine diminishes the eosinopenic effect of cortisone. Further work is in progress.

4. Inquiry on the biogenesis of alkaloids in tobacco plants under Dr. B. C. Bose at the M. G. M. Medical College, Indore.

It was observed in the previous year that leaves can synthetize nicotine and other pyridine alkaloids from inorganic nitrogenous compounds, such as potassium nitrate and ammonium sulphate. Study of the effect of different amino acid precursors, such as tryptophane, proline, arginine, ornithine and anthranilic acid has revealed that tryptophane can effect maximum synthesis of nicotine in tobacco leaves and then the others follow in the above order. Comparative study of nicotine synthesis in roots and stems has shown that roots can synthetize more nicotine from tryptophane than stems. Thiamine and riboflavin when added to tryptophane increased its conversion to nicotine. Spraying of tryptophane on growing plants in the field increased the nicotine content of the leaves to an appreciable extent.

TABLE I.

Showing the effect of potassium nitrate and ammonium sulphate on the synthesis of nicotine in tobacco roots and stems. The value represent the nicotine content in g./100 g. tissue.

Nature of the tissue	Nature of the substrate	Dose of substrate							Per cent increase at peak values
Roots	Potassium nitrate or ammonium sulphate	nil (control) nil (control)	$5M10^{-6}$ $2{\cdot}5M10^{-6}$	$M10^{-5}$ $\cdot 5M10^{-5}$	$5M10^{-5}$ $2{\cdot}5M10^{-5}$	$M10^{-4}$ $\cdot 5M10^{-4}$	$2M10_4{}^4$ $M10^-$	... ...	... ...
		·3	·3	·3	·48	·48	·48	...	60
Stems	Potassium nitrate or ammonium sulphate	nil (control) nil (control)	$5M10^{-6}$ $\cdot 5M10^{-6}$	$M10^{-5}$ $\cdot 5M10^{-6}$	$5M10^{-5}$ $2{\cdot}5M10^{-5}$	$M10^{-4}$ $\cdot 5M10^{-4}$	$2M10^{-4}$ $M10^{-4}$	$5M10^{-4}$ $2{\cdot}5M10^{-4}$	... ...
		·06	·06	·06	·09	·09	·09	·06	50

EFFECT OF AMMONIUM AND NITRATE IONS ON THE SYNTHESIS OF NICOTINE IN TOBACCO ROOTS AND STEMS.

In continuation of last year's work on the effect of potassium nitrate and ammonium sulphate on the synthesis of nicotine in tobacco leaves by *in vitro* culture technique already reported, in the present investigations, the effect of ammonium and nitrate ions from the above sources was studied on tobacco roots and stems. Concentration of potassium nitrate and ammonium sulphate was prepared in such a way that their nitrogen equivalent remained the same and for such equalization of nitrogen each molar concentration of potassium nitrate was equivalent to half molar concentration of ammonium sulphate. It was noted that in the roots and stems also the inorganic nitrogenous sources effected the synthesis of nicotine to a sufficient extent but confirmed the previous finding that the leaf is a better site of synthesis than roots and stems. It was also observed that nitrogen from both the sources was utilized to the same extent for nicotine synthesis. The results are presented in Table I.

COMPARATIVE EFFECT OF ARGININE AND PROLINE ON THE NICOTINE SYNTHESIS IN ROOTS AND STEMS.

One g. slices of roots and stems were cultured in 1 mg. dose of arginine and proline for 24 to 48 hours and the nicotine content determined after completion of incubation. Result of these experiments presented in Table II indicate that the synthesis of nicotine is greater in roots than in stems and greatest in leaves. *Ind. Jour. Med. Res.* **44,** January, 1956.

TABLE II.

Showing the nicotine synthesis in tobacco roots and stems by arginine and proline.

Substrate.	Period of incubation, hours.	ROOT.		STEM.	
		Nicotine content in g./100 g. tissue.	Per cent increase.	Nicotine content in g./100 g. tissue.	Per cent increase.
Nil (control) ...	...	0·24	...	0·100	...
Arginine	24	0·30	25	0·110	10
,, ...	48	0·32	33·3	0·125	25
Nil (control) ...	...	0·24	...	0·055	...
Proline	24	0·28	16·6	0·060	9
,, ...	48	0·28	16·6	0·060	9

EFFECT OF SPRAYING OF THE TOBACCO LEAVES BY ARGININE ON THE SYNTHESIS OF NICOTINE.

In last year's experiments it had been observed that the daily spraying of the growing tobacco plants by tryptophane appreciably increased the nicotine content of the leaves. In the year under review the effect of spraying of arginine was studied. A number of tobacco plants of three months age were divided in two groups and the leaves daily sprayed with arginine solutions containing 10 μg. and 50 μg. doses, respectively. After two weeks, the leaves of the two groups of treated, and of untreated plants were analysed for their nicotine content. The results are presented in Table III.

It was observed that spraying of arginine increased the nicotine content to some extent but the increase is much less than that effected by tryptophane.

TABLE III.

Showing the effect of spraying tobacco plants by arginine on nicotine synthesis in leaves.

Duration of experiment, weeks	Dose of arginine /day/plant, μg.	Nicotine content in g./100 g. leaves	Per cent increase
2	*Nil*	0·10	...
	10	0·10	*Nil*
	50	0·11	10
4	*Nil*	0·10	...
	10	0·11	10
	50	0·12	20

STUDY OF TRANSMETHYLATION OF NICOTINE-NORNICOTINE.

The two wild varieties nicotiana glutinosa and glauca contain sufficient amount of nornicotine and anabasine in addition to nicotine as active principle. Nornicotine is a demethylated product of nicotine. It is not yet clearly known whether nornicotine arises from demethylation of nicotine or it acts as a precursor for the synthesis of nicotine. The present investigation is aimed at studying this aspect of transmethylation in the various regions of these two tobacco plants.

EXPERIMENTAL.

Micro-method for the estimation of nornicotine.—A qualitative test for nornicotine based on the production of a cherry red colour in the presence of quinhydrone solution is reported in the literature by Fienstein and McCabe (1950). In the present investigation it has been possible to suitably modify this test for the quantitative estimation of nornicotine (*Ind. Jour. Med. Res.*, January, 1956). The method is detailed below:—

Nornicotine solutions of different concentrations varying from 10 μg. to 100 μg. per c.c. were prepared and to each 5 c.c. of these solution, 5 c.c. of phosphate buffer of pH 7 and 10 c.c. of 0·2 c.c. aqueous quinhydrone solution were added. A cherry red colour gradually developed. The intensity of this colour was found to be proportional to concentrations within the range of 10 μg. to 100 μg. of nornicotine per c.c. Thus, by the use of quinhydrone reagent it was possible to measure 10 μg. of nornicotine per c.c. This new method, therefore, is sufficiently accurate for micro-estimation of nornicotine and it is felt that by using a more sensitive galvanometer, it will be possible to assay even smaller quantities of nornicotine.

Per cent recovery of nornicotine.—For the evaluation of the above the following alkaloidal extracts were prepared:—

(*a*) Two g. of dry tobacco leaves were taken and total aklaloids extracted by grinding it with sodium-hydroxide solution and then extracting with chloroform. The chloroform solution was then agitated with successive portions of dilute sulphuric acid and the aqueous layer containing the sulphates of alkaloids separated. This was made alkaline with ammonium and the free alkaloids finally extracted with chloroform. The chloroform was then carefully evaporated on a water-bath. The extract was made to 25 c.c. with distilled water.

(*b*) Batches of 1 g. of the same samples of dry tobacco leaves were then taken and to these 10 mg. of nornicotine added. The total alkaloids were then extracted in the same way as above, and the extract made up to 25 c.c. with distilled water. This extract was further diluted to different volumes keeping the expected range of concentration of nornicotine up to 10 μg. per c.c. Colour reaction for nornicotine was then developed in these diluted extracts and also in the extract (*a*) and from the per cent transmittance readings of the samples and standard nornicotine solutions, the per cent recovery of the added nornicotine evaluated. These were found to range within a small limit of 88·7 to 92·2 per cent with an average value of 89·4 per cent as presented in Table IV:—

TABLE IV.

Batch	Amount of dry tobacco leaf, g.	Quantity of nornicotine added, mg.	Nornicotine estimated per g. of leaf, mg.	Per cent recovery of added nornicotine
A.	2	*Nil*	2·31	*Nil*
B.	1	10	11·25	89·4

The results indicate that the method can be easily applied for micro-estimation of nornicotine in presence of nicotine.

STUDY OF THE INTERCONVERSION OF NICOTINE-NOR-NICOTINE.

With the help of the above method the process of transmethylation involved in the interconversion of nicotine-nornicotine was studied with a view to ascertain as to whether nicotine is the precursor for nornicotine or vice versa. In the presence of a methyl group acceptor and an enzyme system, nicotine acts as a methyl donor and is converted to nornicotine.

The enzyme preparation required for this investigation, and the procedure of incubation are detailed below:—

Preparation of enzyme.—Ten g. of tissue (root, stem and leaf) of *N. tabacum* and *N. glauca* were thoroughly ground with 30 c.c. to 35 c.c. of water and the extract allowed to stand for two hours. It was then filtered and the filtrate poured slowly in cold acetone with constant stirring. By this process, the enzyme precipitated out and it was then dried in vacuum after decanting off the acetone. Emulsion of this enzyme was made in 25 c.c. water and this was preserved in the refrigerator for use in later investigations.

Procedure of incubation.—To a series of conical flasks, containing 5 mg. of nicotine in 5 c.c., 5 mg. ethanolamine or guanido acetic acid as methylacceptor in 5 c.c., 5 c.c. phosphate buffer of pH 7, and 30 c.c. liquid medium (magnesium sulphate 0·2 g., potassium chloride 0·2 g., Cal. dihydrogen phosphate 0·1 g., sod. sulphate 0·1 g. and sucrose 10 g. in 1,000 in distilled water), were added 5 c.c. of the aqueous enzyme preparation equivalent to 2 g. of leaf, root or stem from both *N. tabacum* and *N. glauca.* These flasks were then incubated for 48 hours at 37°C. after which the contents were analysed for nornicotine. For each set of enzyme preparations three conical flasks for both ethanolamine and guanido-acetic acid and also another control set in which the ethanolamine and guanido acetic acid were replaced by water were arranged. It was noted that in control set no nornicotine was developed thus showing that the enzyme preparation was free from any methyl acceptor. The results are shown in Table V:—

It would thus appear that the leaf, root and stem of both *N. glauca* and *N. tabacum* contain an enzyme system which can demethylate nicotine to nornicotine in presence of the methyl acceptor ethanolamine. It was further noted that per g. of tissue, *N. glauca* contains much higher contents of the enzyme than *N. tabacum.* While comparing the capacity of individual tissue in each of these two plants, in both the cases leaf was found to contain higher activity than root and stem. It was also noted that incubation of nicotine in the presence of guanido-acetic acid did not give rise to any nornicotine by any tissue enzyme extract from either of the two plants.

Methylation of nornicotine to nicotine.—The experiment was arranged in an identical manner used in the study of demethylation described before, except that nicotine was replaced by a standard solution of nornicotine and the methyl acceptors ethanolamine and guanido-acetic acid replaced by the methyldonating compound methionine. On completion of the incubation period of 48 hours, the contents of the flasks were analysed for nornicotine and it was observed that no methylation of nornicotine to nicotine had taken place. The observations of the control set as well as the methionine sample also showed no change from the initial standard of nornicotine content. The results are shown in Table VI.

From the survey of results presented above, it was observed that all the three main regions of nicotine to nornicotine plants contain an enzyme system which can demethylate nicotine in presence of ethanolamine but the methylation of nornicotine to nicotine in presence of methionine was not observed. Further work is in progress under various conditions of concentration and pH of the different methyl donors and acceptors.

TABLE V.

Showing the demethylation of nicotine to nornicotine by enzyme preparation of leaf, root and stem of N. glauca *and* N. tabacum *in presence of methyl group acceptors.*

Name of the plant	Source of enzyme.	Amount of nicotine incubated, mg.	Amount of methyl group acceptor, mg.	Nornicotine formed, mg.	Per cent conversion of nicotine to nor nicotine.
N. glauca	Leaf	5	Ethanolamine 5	1·65	33·0
	Root	5	„	1·16	23·2
	Stem	5	„	1·16	23·2
N. glauca	Leaf	5	Guanido-acetic acid 5	*Nil*	*Nil*
	Root	5	„	*Nil*	*Nil*
	Stem	5	„	*Nil*	*Nil*
N. tabacum	Leaf	5	Ethanolamine 5	1·10	22·0
	Root	5	„	0·80	16·0
	Stem	5	„	0·80	16·0
N. tabacum	Leaf	5	Gaunido-acetic acid 5	*Nil*	*Nil*
	Root	5	„	*Nil*	*Nil*
	Stem	5	„	*Nil*	*Nil*

TABLE VI.

Showing the methylation of nornicotine to nicotine by enzyme preparation of leaf, root and stem of N. glauca *and* N. tabacum *in presence of methionine.*

Name of plant	Source of enzyme	Amount of methionine taken, mg.	AMOUNT OF NORNICOTINE		Per cent of nicotine formed
			Before incubation, mg.	After incubation, mg.	
N. glauca ...	Leaf Root Stem	5, 2 and 1	1	1	*Nil*
N. tabacum ...	Leaf Root Stem	5, 2 and 1	1	1	*Nil*

5. Inquiry on the action of general anaesthetic agents on the automaticity of the atrioventricular node under Dr. R. B. Arora at the Sawai Man Singh Medical College, Jaipur.

PART I.

The mechanism of action of thiopentone, kemithal and evipal on the cardiovascular system was studied and the role of autonomic agents in potentiating or antagonizing the deleterious effects was elucidated. Experiments were conducted in 107 anaesthetized dogs having either S-A or A-V rhythms, and in seven heart-lung preparation of dogs. Thiopentone in anaesthetic concentrations was found to reduce cardiac rhythmicity more so of A-V node than of S-A node. Cardiac output may be diminished and venous pressure raised. For the first ten minutes after thiopentone administration cardiac sensitivity to vagal stimulation and acetylcholine was diminished. But continuous acetylcholine infusion enhanced the cardio-inhibitory and hypotensive effects of thiopentone. Thiopentone was found to antagonize the cardioaccelerator and pressor responses of adrenaline. Administration of sympathomimetics was found to diminish the deleterious effects of thiopentone. The results indicate the necessity of a routine use of a para-sympatholytic as a premedicant and also establish the resuscitative value of sympathomimetics in cardiovascular catastrophes during barbiturate anaesthesia. An interesting observation was that the depressor response of adrenaline is facilitated by thiopentone.

Preliminary investigations on the effect of ether inhalation on the cardiac rhythmicity and E.C.G. tracings was conducted in seven dogs having either S-A or A-V rhythm. Ether reduced cardiac rhythmicity and reduced P-R interval upto even complete disappearance of P wave.

In 35 anaesthetized dogs a comparative study was made on the physiological aspects of S-A and A-V nodes. It was found that normal inhibitory influences of vagus was less on A-V than on S-A node. A-V node was found to be more responsive to acetylcholine and less so to cardio-accelerators than S-A node.

PART II.

A number of indigenous drugs and some recently synthesized compounds related to procaine were investigated for their antiarrhythmic activity. Preliminary tests on the refractory period of isolated rabbit auricles showed that N. jatamansi (an Indian indigenous drug) and a new local anaesthetic, McN-100 warrant further experimental trials. Antiarrhythmic activity of these drugs was investigated in (i) (a)—acetylcholine and (b) aconitine-induced auricular fibrillation, (ii) auricular flutter produced by injury-stimulation procedure and (iii) ventricular arrhythmias produced by (a) ouabain, (b) adrenaline and (c) hydrocarbon-adrenaline in dogs. The effects on the refractory period and conduction time were studied from E.C.G. tracings in cats. Quinidine was used as standard for comparison. In all 163 experiments were performed (five to ten experiments per drug with each technique).

(1) N. jatamansi was found to be less potent antiarrhythmic than quinidine. But it scores significant advantages over the latter

drug in exerting less marked myocardial depressant action, relatively less effect on conduction and low toxicity.

(2) McN-100 was less effective in auricular fibrillation more effective in auricular flutter and equivalent to quinidine in ventricular arrhythmias. It, however, was superior to quinidine in having less effect on conduction and possessing lower toxicity.

The results obtained indicate that the above two drugs may prove useful clinically in cardiac arrhythmias.

DETAILED NOTE.

Inquiry on " Action of general anaesthetic agents on automaticity of A-V node ".

Section A:—Automaticity of A.V node.

Experiments were conducted on 35 Mongrel dogs with intact heart having either S-A or A-V node responsible for the automaticity.

1. *Adrenaline HCl and nor-adrenaline.*—Tachycardia after adrenaline in A-V nodal rhythm was much less as compared to seen in S-A nodal rhythm. In A-V nodal rhythm sometimes P wave reappeared in E.C.G. tracings for a short time after administration of 4 mcgm./Kgm. adrenaline or nor-adrenaline.

2. *Acetylcholine chloride.*—The initial phase of bradycardia, seen with higher doses of acetylcholine, was more often seen with A-V nodal rhythm than S-A nodal rhythm. This initial bradycardia was seen with comparatively lower doses and was more in degree in A-V nodal rhythm than in S-A nodal rhythm.

3. *Atropine Sulph.*—Tachycardia after atropinisation was comparatively much less in A-V nodal rhythm than seen after S-A nodal rhythm. Atropine when given in A-V nodal rhythm sometimes resulted in the appearance of P wave in E.C.G. tracing which lasted only for a very few minutes.

Section B.—Ultra-short acting barbiturates.

1. *On the automaticity of S-A and A-V nodes.*—Experiments were carried out on 55 dogs with intact heart having either sino-auricular or auriculo-ventricular node responsible for the automaticity.

(i) Thiopentone, kemithal and evipal have been found to have two paradoxical effects on cardiac rate, accelerator or inhibitor, and widely variable effect on blood pressure. With increase in dosages the accelerator response became rarer and the inhibitor response commoner. On an average, evipal was found to have predominantly accelerator effect, and thiopentone and kemithal inhibitor effect. In S-A nodal rhythm the accelerator response was more often seen than in A-V nodal rhythm.

(ii) Thiopentone and kemithal had more pronounced hypotensive effect than evipal. During the injection of thiopentone and kemithal, or afterwards, sometimes there was a slight transient rise in blood pressure. Traube-Hering waves if present before, disappeared after the barbiturates to reappear in some instances after varying periods.

(iii) In cases of ' upper nodal ' type of A-V nodal rhythm thiopentone sometimes shifted the site of origin of impulse from upper nodal to mid nodal area.

2. *Thiopentone and parasympathetic nervous system:*

(i) *Relation to vagus*—(a) It was observed that thiopentone when given after vagotomy did not increase heart rate, it consistently resulted in bradycardia.

The two divergent types of effects on the cardiac rate, as seen in dogs having intact vagi were, as seen before, dependent on the initial heart rate which is roughly an index of vagal tone. In dogs with high vagal tone thiopentone produced tachycardia and in the absence of vagus or vagal tone it produced bradycardia.

(b) Vagotomy had no effect on the cardio-inhibitory effect of thiopentone.

(c) In dogs and cats vagi had been sectioned in the neck and the peripheral ends were stimulated for 15 seconds by Grass Model IV stimulator delivering square waves. It was found that percentage fall in heart rate to vagal stimulation was less for the first ten minutes after adminstration of thiopentone than seen before it.

(ii) *Relation to acetylcholine.*—

(a) It was observed that percentage fall in heart rate due to acetylcholine was more if given before than within first ten minutes after administration of thiopentone. It was also observed that the hypotensive effect of acetylcholine was slightly more if injected after administration of thiopentone.

(b) In eight vagotomized dogs thiopentone 10 mgm./kgm. was administered intravenously first during continuous acetylcholine infusion at the rate of 0·01 to 0·03 mcgm./kgm./min. and second after atropinization. It appeared that cardio-inhibition due to thiopentone was mostly increased but in some cases uninfluenced by acetylcholine infusion. The hypotensive effect of thiopentone was also consistently more prolonged when given during acetylcholine infusion.

3. *Thiopentone and sympathomimetics:*

(i) *Effect of adrenaline (or nor-adrenaline) after thiopentone administration.*—In four out of five experiments it was observed that the chronotropic effect of adrenaline was less marked in degree and duration after prior administration of thiopentone than seen before it, the maximum being within one minute of thiopentone injecton. The pressor response of adrenaline was found to be less when it was given after administration of thiopentone than seen before it. In four experiments adrenaline manifested a depressor response following initial pressor effect. In these dogs when adrenaline was given after thiopentone administration, it was observed that though the fall in blood pressure due to adrenaline was less in extent, but was much more in duration. In two experiments it was observed that when adrenaline 20 to 40 mcgm. was given in the course of one minute it produced a fall of blood pressure. In these experiments the same dose when it was given after thiopentone administration, the depressor response of adrenaline was more prolonged.

In the experiments on A-V rhythm injection of adrenaline caused a temporary return of P wave. This phenomenon was absent when the same dose of adrenaline was given after thiopentone administration.

(ii) *Effect of thiopentone and adrenaline (or nor adrenaline) administered together.*—On an average, adrenaline diminished the cardio depressant effect of thiopentone slightly in degree, but had no significant effect on duration of depression. Adrenaline also diminished the hypotensive effect of thiopentone.

(iii) *Effect of thiopentone during continuous adrenaline (or nor-adrenaline infusion.*—Adrenaline infusion consistently prolonged the duration of hypotension due to thiopentone. On stopping the adrenaline infusion, in four experiments it was observed that there was a rapid rise of blood pressure, which was sustained at a lower level during the period of infusion.

(iv) *Thiopentone and adrenaline in heart-lung preparation of dogs.*—Heart-lung preparation in dogs was carried out by the technique of Starling and Peterson as modified by Krayer and Mendez.

Observation recorded were (i) arterial-pressure from brachiocephalic artery, (ii) cardiac output by the help of Weese stromuhr, (iii) venous pressure from inferior vena cava and (iv) heart rate from E.C.G. tracings.

It was found that thiopentone 1 mgm./kgm. had no effect on cardiac output, cardiac rhythmicity, arterial pressure and venous pressure. Thiopentone 2 mgm./kgm. caused, on an average, 15 per cent reduction of cardiac output, five per cent reduction in heart rate, ten per cent fall in blood pressure and 4 mm. rise in venous pressure. After the initial changes cardiac functions improved but did not come back to normal for a period of 15 to 20 minutes.

Continuous adrenaline infusion at the rate of 0·05 mcgm./kgm./min. improved the cardiac output by ten per cent, raised the blood pressure by five per cent and diminished the venous pressure by 5 mm. of water but had no significant effect on heart rate. When thiopentone was administered during continuous adrenaline infusion the reduction in cardiac efficiency was slightly more in degree but the absolute values of cardiac output, venous pressure and arterial pressure did not exceed the figures seen with the same dose of thiopentone when given without adrenaline infusion.

Section B.—Ether.

In preliminary investigation of the effect of ether inhalation on the junctional tissues of heart was studied in seven dogs. Inhalation was given at the rate of 0·1 to 0·5 c.c./kgm./min.for five minutes. On S-A as well as A-V nodal rhythms the inhalation of 0·2 c.c./kgm./min. and more caused reduction in heart rate and fall in blood pressure. The interesting finding was that in S-A nodal rhythm ether caused a gradual reduction in P-R interval usually resulting in disappearance of P wave.

PART II.

Inquiry on " Study of antiarrhythmic activity of eight recently synthetized compounds on experimentally produced cardiac arrhythmias in intact hearts of mammals and in " Heart-lung preparation ' of dogs ".

In investigating the antiarrhythmic drugs, a variety of procedures were employed for the producton of experimental arrhythmias in different species of animals in order to give more confidence to the results and their interpretations.

Section A—Indigenous drugs:

Jatamansi: In this laboratory, while investigating the antifibrillatory action of drugs selected from a variegated confetti of indigenous medicaments, it was noticed that Nardostachys jatamansi (N.O. Valerianaciae) or the Indian Nard has been extensively used since times immemorial (800 B.C.) for a diversified group of cardiac maladies. Tests were, therefore, made on the refractory period of isolated rabbit auricles. Drug showed sufficient promise to warrant further experimental trials and hence its antiarrhythmic actions were explored in detail. In addition, LD_{50} in albino mice was worked out.

Active principle of N. jatamansi, volatile oil, especially made fit for intravenous use was used throughout and comparison was made with quinidine.

1. In tests on the refractory period of isolated rabbit auricles, N. jatamansi brought about 6·1 per cent reduction in maximum frequency (quinidine—13 per cent).

2. N. jatamansi (both in innervated and decentralized hearts) caused reversion to normals sinus rhythm in six out of ten experiments at an average dose of 14 mgm./kgm. (quinidine—8 mgm./kgm.).

3. In aconitine-induced auricular fibrillation N. jatamansi failed to bring about the arbitrary 'end point' (1:1 rhythm with heart rate below 200 beats per minute) when given upto 35 mgm./kgm. (Quinidine—8 mgm./kgm.).

4. Percentage reduction in the duration of acetylcholine induced auricular fibrillation was 46 with quinidine at 3·5 kgm./mgm. while N. jatamansi did not have any effect at this dosage. However, at 12·5 mgm./kgm. it brought about 66 per cent reduction in the duration of auricular fibrillation; and at 15 mgm./kgm. it terminated the fibrillation within 1 minute 30 seconds of the intravenous injection.

5. Ten mgm./kgm. of jatamansi given intravenously caused only a transient fall of blood pressure in anaesthetized cats while quinidine at the same dosage had marked hypotensive effect.

6. N. jatamansi caused on an average 20 per cent increase in conduction time (P-Q interval) and 15 per cent increase in refractory period (Q-T interval), while the same figures for quinidine (Arora and Madan, 1956) were 46 per cent and 22 per cent, respectively.

Jatamansi failed to arrest the digitalis-induced ventricular arrhythmias when given upto a dosage of 35 mgm./kgm.

Toxicity in mice.—LD_{50} of jatamansi was found out to be 80·3 mgm./kgm. while in case of quindine it was 55 mgm./kgm.

Section B.—Synthetic compounds:

(i) McN-100—A new local anesthetic was investigated for its anti-arrhythmic action. Methods employed have already been mentioned. Comparison was made with quinidine and procaine amide. The results obtained showed that—Tridiurecaine reverted auricular flutter in all the six experiments at an average dose of 14 mgm./kgm. and quinidine at 21 mgm./kgm.

1. (a) *Action on aconitine-induced atrial fibrillation.*—With tridiurecaine the reduction of auricular rate and increase in ventricular rate was noted and 1:1 rhythm could be established when the total dosage administered intravenously reached 70 mgm./kgm. The rate however, did not become less than 200 per minute. With quinidine the 'end point' was reached at 18 mgm./kgm.

(b) *Action on acetylcholine-induced atrial fibrillation.*—Tridiurecaine was apparently almost equally effective as quinidine when each was administered in 2·5 mgm./kgm. dose.

2. *Action on hydrocarbon-adrenaline-induced ventricular arrhythmias.*—Tridiurecaine and quinidine exhibited preventive action on an average dosage of 7·5 mgm./kgm. and 5 mgm./kgm., respectively.

3. *Action of tridiurecaine on refractory period.*—As shown by the experiments on isolated rabbit auricles, quinidine was found superior to tridiurecaine in increasing the refractory period.

4. *Action on electrocardiogram.*—The data of the action of tridiurecaine and quinidine on P-Q (conduction time) and Q-T (refractoriness) intervals in cats showed that there is a significant difference in the effect of the two drugs. Tridiurecaine is better than quinidine from the point of view of P-Q interval though the increase in refractory period was more noted with quinidine.

Section C.—Ventricular perfusion:

Central action of Nardostachys jatamansi.—In order to investigate the central action of jatamansi, ventricular perfusion experiments were performed in five anaesthetized dogs by employing the method of Dixit, B.B. (as demonstrated in Indian Council of Medical Research meeting, 1955).

Results obtained indicated that Nardostachys jatamansi exerted a slight central depressant action on medullary centres.

6. Inquiry to determine the chemical configuration of cinchona alkaloids responsible for its antiveratrinic action under Dr. R. B. Arora at the Sawai Man Singh Medical College, Jaipur.

A. CINCHONA ALKALOIDS AND CUPREINES.

One hundred and eighty-seven experiments were carried out on the determination of antiveratrinic activity of epiquinine, epicinchonine quinuclidine, quinicine, lepidine, ethyl ether cupreine and propyl cupreine.

The positive results of these experiments along with the results of some connected compounds reported last year are given in Table No. 1.

B. CHLOROQUINE COMPONENTS.

One hundred and sixty one experiments were carried out on the determination of the antiveratrinic activity of the chemical components of chloroquine i.e. 4-amino-quinoline, 7-chloro-quinoline, diethylamine, 4-diethyl-amino-methyl-l-butyl amine and deoxy-cinchonine, deoxycinchonidine, 9-chloro-deoxycinchonine, 9 chloro-deoxy-cinchonidine.

The positive results of these experiments are given in Table No. II.

TABLE I.

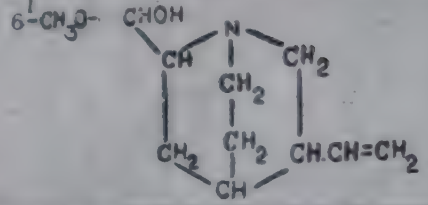
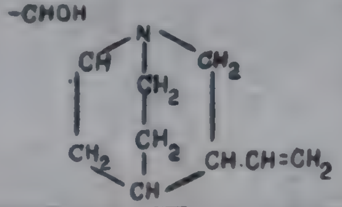
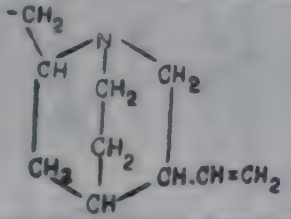
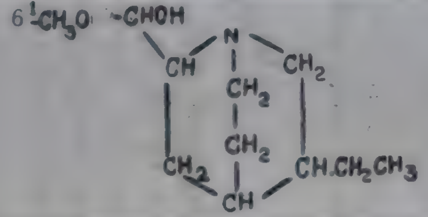
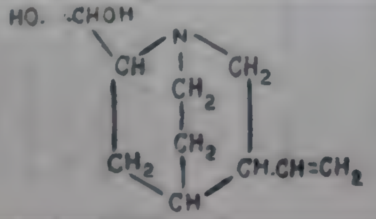
Chemical Formula	Rabe's nomenclature	Name	Direction of rotation			(a)	D	Min. conc. which prevents A.V. action
			C³+C⁴	C⁸	C⁹			
		Quinine	+	—				1:100,000
			(+—)		—	—158	·2°	
	6′-methoxy-3-vinyl ruban-9-ol	Quinidine	+	+				1:100,000
			(+—)		+	+24	·5°	
		Epiquinine	+	—	+	+43		1:100,000
		Cinchonine	+	+	+	+224	·3°	1:300,000
			(+—)					
	3-vinyl-ruban –9–ol				+	—111	·4°	
		Cinchonidine	+	—				1:300,000
			(+—)					
		Epicinchonine	+	+	—	—120	·0	1:300,000
						+179		
		Deoxycinchonine	+				·3°	1:50,000
			(+—)	+	*Nil*			
	3-vinyl-ruban	Deoxycinchonidine	+					1:50,000
			(+—)	—	*Nil*	—29	·9°	
	6′-methoxy-3-ethyl ruban-9-ol	Dihydroquinidine	+	+	+	+237	·5°	1:30,000
	6′-hydroxy-3-vinyl ruban 9-ol	Cupreine				—175	·5°	1:30,000

TABLE I.—(Concld.).

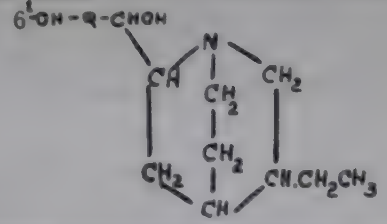
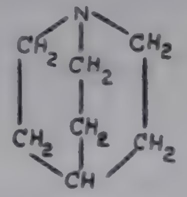
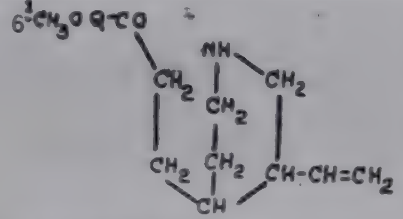
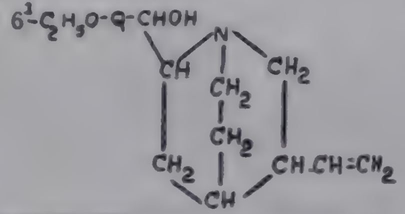
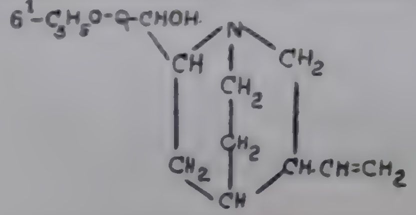
Chemical formula	Rabe's nomenclature	Name	Direction of rotation			(a)	D	Min. conc. which prevent A.V. action
	6′-hydoxy-3-ethyl ruban-9-ol	Dihydrocupreine				155	·5°	1:10,000
		Quinuclidine						No action even in 1:10,000
		Quinotoxine						1:100,000
	6′-ethoxy-3-vinyl ruban-9-ol	Ethyl cupreine						1:300,000
	6′-propyl 3-vinyl ruban-9-ol	Propyl-cupreine						1:1,000,000

TABLE II.

Name of the compound	Veratrine response produced by	OBSERVATIONS ON PREVENTION OF VERATRINE RESPONSE		OBSERVATIONS ON ABOLITION OF VERATRINE RESPONSE		
		Number of positive experiments	Conc. which prevents the response	Number of positive experiments	Conc. which abolishes the response	Average time taken in minutes
4-amino quinoline …	Veratridine in conc. of 10^{-7}	5 out of 5	$3 \cdot 3 \times 10^{-5}$	3 out of 3	$3 \cdot 3 \times 10^{-5}$	9
7-chloro-quinoline …	,, ,, ,,	5 out of 5	10^{-5}	4 out of 4	10^{-5}	24
Diethylamine …	,, ,, ,,	*Nil*	No action even in 10^{-4}	*Nil*	No action even on 10^{-4}	…
4-Diethylamino-methyl-1-butylamine …	,, ,, ,,	4 out of 5	10^{-4}	3 out of 3	10^{-4}	8
Deoxy-cinchonine …	,, ,, ,,	5 out of 5	2×10^{-5}	4 out of 4	2×10^{-5}	2
Deoxy-cinchonidine …	,, ,, ,,	6 out of 6	2×10^{-5}	3 out of 4	2×10^{-5}	42
9-chloro-deoxy-cinchonine	,, ,, ,,	3 out of 4	$3 \cdot 3 \times 10^{-6}$	4 out of 5	$3 \cdot 3 \times 10^{-6}$	30
9-chloro-deoxy-cinchonidine	,, ,, ,,	4 out of 4	$3 \cdot 3 \times 10^{-6}$	4 out of 6	$3 \cdot 3 \times 10^{-6}$	20
Quinine … …	,, ,, ,,	4 out of 6	10^{-5}	3 out of 3	10^{-5}	14
Quinidine … …	,, ,, ,,	5 out of 7	10^{-5}	3 out of 3	10^{-5}	14

C. SOME OTHER COMPOUNDS WITH DIFFERENT CHEMICAL STRUCTURE.

Drugs like procaine, procaineamide, chloropromazine, McN-100, McN-29-A-11 and dilantin were also investigated for their antiveratrinic action. The results obtained indicated that all these compounds (except for dilantin) were qualitatively similar to quinine and quinidine in their action on veratrine response. Dilantin, however, does not prevent the development of veratrine response but abolishes it in a concentration of 1:10,000 in three minutes.

D. ANTIARRHYTHMIC ACTIVITY.

Chloroquine and amodiaquin when investigated in experimental auricular flutter (innervated and decentralized hearts) indicated that the doses required were a little less than one third the dose necessary for quinidine to revert the flutter to normal sinus rhythm. Both the drugs showed superiority over quinidine and procaine amide in acetylcholine induced as well as aconitine-induced auricular fibrillation.

Chloroquine increased the conduction time less and the refractory period was found to be increased more than with quinidine. Amodiaquin increased the conduction time more but the refractory period less with quinidine.

Amodiaquin had no preventive action against either hydrocarbon-adrenaline induced or adrenaline induced ventricular arrhythmias. The effects of causing fall in blood pressure were the same with chloroquine and quinidine, amodiaquin, however, had less pronounced effect.

It is suggested that this newly found out antiarrhythmic property of chloroquine and amodiaquin be put to clinical trials. Pamaquin was found stronger than quinidine in atrial fibrillation and atrial flutter. It caused greater lengthening of refractory period and conduction time than quinidine.

Quinidine was more effective in reverting the abnormal ventricular ectopic activity to normal rhythm than procaine-amide when Harris technique was employed.

PART II.

" A study on the chemical structure of cinchona alkaloids and cupreines responsible for its negative Ionotropic action in isolated Amphibian and Mammalian hearts."

Sixty experiments were conducted on isolated perfused amphibian and mammalian hearts to establish the chemical configuration of Cinchona alkaloids and cupreines responsible for its negative ionotropic action.

It was observed that like the cardiac glycosides and aglycones (Arora and Krayer—1952 and Arora, 1953). The cardiac activity of cinchona alkaloids and cupreines may be based on the same sterochemical and chemical requirements as its antiveratrinic activity (Arora, 1955, and Arora and Dandiya to be published).

7. Inquiry into the anti-diabetic properties of some selected indigenous plants under Dr. (Smt.) Ranita Aiman at the B. J. Medical College, Poona.

The studies were undertaken to determine whether the indigenous anti-diabetic drugs *Caseria esculenta* (root) and *Bassia longifolia* (bark) exert an insulin-like action or had any other effect on the blood sugar of normal animals or animals made diabetic by alloxan or pancreatectomy. Drug administration was in some cases carried out over a prolonged period as advocated in Ayurveda. The work was carried out on rabbits and dogs.

(I) STUDIES ON NORMAL RABBITS.

(*i*) *Caseria esculenta.*—Control fasting blood-sugar estimations were done on six normal rabbits as follows:—

(*a*) Every hour five hours without dosage of any kind.

(*b*) On simple insertion of stomach-tube before and every ½, 1, 2, 3, 4, and 5 hours thereafter.

(*c*) On administration of distilled water by stomach-tube before and every ½, 1, 2, 3, 4, and 5 hours thereafter.

These rabbits were then given powdered root of *Caseria esculenta* in various doses as hot and cold water extracted by stomach-tube and examinations of blood-sugar were done periodically as above and the results compared.

(*ii*) *Bassia longifolia.*—The above studies (I, *i*) were repeated on another batch of four rabbits, *Bassia longifolia* was used as cold water extract of powdered bark.

(*iii*) After a lapse of some days these drugs were crossed over so that in all 10 rabbits were studied for (*i*) and six for (*ii*).

METHODS OF EXTRACTION.

(1) *Cold water extract.*—The calculated amount of the powdered drug was soaked in the measured amount of distilled water for 18 hours and strained through a layer of cloth. The amount of distilled water corresponded with that given previously by stomach-tube in the control experiment.

(2) *Hot water extract.*—The calculated amount of the powdered drug was put in 125 c.c. to 150 c.c. of distilled water and boiled down to the required volume.

Neither of these drugs appeared to have any effect on the blood-sugar levels of normal rabbits.

(II) STUDIES OF NORMAL DOGS.

(*i*) *Caseria esculenta.*—Two normal dogs were given the measured dose of this drug orally over a prolonged period of 1½ to 6 months and fasting blood-sugar estimations and dextrose tolerance curves were done before and during administration of the drug.

(*ii*) *Bassia longifolia.*—This was studied as in (II, *i*).

Neither of these drugs appeared to have an effect on the blood-sugar levels or on the dextrose-tolerance curves of normal dogs.

(III) STUDIES ON ALLOXAN DIABETIC RABBITS.

Five rabbits were given cold water extracts of *Caseria esculenta* by stomach-tube daily for varying periods and 2 hours before an intravenous injection of a diabetogenic dose (200 mg./kg. body-weight) of alloxan monohydrate. Blood-sugar studies were done periodically at $\frac{1}{4}$,$\frac{1}{2}$, 1, 2, 4, 6, 8, 24, 48 and 72 hours. The drug was also continued during the post-alloxan period.

Two rabbits were used as control. They were not given any drug but were given the same diabetogenic dose of alloxan intravenously and blood-sugar estimations done periodically as above and the results compared.

From the blood-sugar data collected it appears as if the first hyperglycæmic phase of alloxan diabetes which is supposed to be due to stimulation of liver glycogenolysis was not makred. The average rise in blood-sugar taking the fasting level as 100 per cent was as follows for the two groups:—

	per cent
Control	187
Drug-treated group	125

Average time for the 2nd hypoglycæmic phase to develop was 3 hours in the control group and 2·3 hours in the drug-treated group.

The average blood-sugar levels at 24 and 48 hours for the two groups were as follows:—

	per cent
Control	235 and 389
Drug treated group	65 and 252

It appears as if the final hyperglycæmia was delayed in the drug-treated group.

Two of these rabbits died within 24 hours of the alloxan administration and two within 6 days.

(IV) STUDIES ON ALLOXAN DIABETIC DOGS.

(*i*) *Caseria esculenta.*—The drug was administered orally to an alloxan diabetic dog in a constant daily dose with a constant dose of insulin for 13 weeks. During this period the dog was on a weighed diet. Fasting blood-sugar estimations were done periodically. When it had been stabilized the dose of insulin was gradually increased. Blood-sugars were on a higher level than during the control period with a fixed dose of insulin. The general condition of the animal deteriorated and one day the animal collapsed and died suddenly.

Post-mortem examination revealed nothing significant except that the medula of the kidneys appeared pale. Microscopic examination showed central necrosis and congestion of liver, cloudy degeneration of the kidney and sub-acute gastritis.

It would appear that the cause of death was the effect of alloxan on pancreas and liver.

(*ii*) Two normal dogs were given *Caseria esculenta* daily for a prolonged period in their diet. During this period three dextrose tolerance curves were done on each at regular intervals. They were then given alloxan monohydrate 60 mg./kg. body-weight intravenously as five per cent aqueous solution and blood-sugar examinations were done at intervals of ¼, ½, 1, 2, 4, 6, 8, 24, 48, and 72 hours. Dextrose tolerance curves were done on 12th and 30th day and fasting blood sugar examination on 24th day of alloxan administration, the drug being continued throughout.

One dog was used as a control. No drug was given to this dog. Alloxan was given in the same dosage as above and blood-sugar studies were done as in (IV) (*i*) (*ii*).

(*B*) *Bassia longifolia.*—This was studied as in IV, (*i*), (*ii*).

From the data obtained it would appear that previous administration of these drugs did not protect the dogs from the diabetogenic effects of alloxan nor was the severity of the diabetes reduced.

(V) STUDIES ON DEPANCREATIZED DOGS.

The effect of *Caseria esculenta* is being studied on a depancreatized dog.

8. Inquiry on the study for anthelmintic action of shell oil of cashew-nut (*Anacardium occidentale* Linn.) under Dr. N. V. Bhaduri at the School of Tropical Medicine, Calcutta.

The present work on the shell liquid of cashew-nut (*Anacardium occidentale* Linn.) was carried out in collaboration with Dr. R. N. Chakravarty, Professor of Chemistry, in order to evaluate the anthelmintic action of the whole shell extract as also of its chemical components against the various intestinal helminth parasites of man. The cashew-nuts were obtained from Contai (Dist. Midnapore, West Bengal) where the plants grow wild.

The prepared shell liquid of cashew-nut is an acrid oily dark brown, viscous liquid and irritant to the skin and mucous membrane. The extract contains (*i*) anacardic acid—90 per cent; (*ii*) cardol—8 to 10 per cent; and (*iii*) a varying amount of cardanol depending upon mode and duration of heating during the extraction of liquid. Anacardic acid, a substituted salicylic acid (2-hydroxy-6 n-pentadecadienyl benzoic acid) and prolonged heating decomposes it into cardanol (3-pentadecadienyl phenol). The second constituent cardol is a substitutive resorcinol (5-pentadecadienyl phenol).

The study of the anthelmintic action of the prepared whole extract was continued this year. It was used on hospital patients as well as on outdoor patients of the School. As before the extract was put in hard gelatin capsules which were allowed to be swallowed. The patients were prepared with a dose of saline purgative on the previous afternoon and milk diet at night. The drug was given in the morning and a saline purgative was given two hours later. The dosage used was 5 g. to 6 g. for adults, 4 g. for those between 10 and 14 years of age and 2·5 g. to 3·5. g. for children between four and nine years of age. The treatment was repeated, if necessary after a period of 10 days. This regime was used on 71 cases of round-worm infection and 112 cases of hook-worm infection. In the first series 41 cases were completely cured by single treatment and by a second treatment of 15 cases, eight were cured. In the second series (cases with hook-worm infection) 53 were completely cured by a single treatment and six more cases showed very scanty ova on re-examination of the stool (by concentration method D.C.F.) and were considered to be practically cured. And after a second treatment eight out of 22 cases were absolutely cured and one more case practically cured. The drug was used on patients who had, hypertension, kala-azar, malaria, amœbiasis, leukæmia, anæmia, pregnancy, diabetes, nutritional œdema, splenomegaly, apart from helminthiasis and in no instances any untoward symptoms were observed. The drug appears to be a safe non-toxic and well-tolerated anthelmintic.

As the mode of administration of the extract in hard gelatin capsules was not very suitable for children a more convenient method was sought for.

It was found that the extract mixed with milk did not cause much irritation to the skin or mucous membrane and two series of cases of hook-worm and round-worm infections were treated with the drug given in this way. Of 21 cases of *Ascaris* infection six were cured by a single treatment and after a second treatment of eight uncured cases, three were cured. Similar observations were made on 48 cases of hookworm infection when 15 were absolutely cured and one more case practically cured after one treatment and of five uncured cases one more was cured by a second treatment. Thus, it appears that the extract mixed with milk may be administered with convenience but loses much of its anthelmintic value.

This extract was used on cases of *Hymenolepis nana*, *H. diminuta*, *Strongyloides stercoralis* and *Enterobius vermicularis* and was found to be of value.

Against *Trichuris trichiura*, *Tænia saginata* and *T. solium*, the extract did not appear to be effective.

Of the constituents of the extract of cashew-nut shell cardol showed a chemical composition very similar to the reputed anthelmintic hexylresorcinol and this fraction was chosen for study of its anthelmintic properties.

Stadeler (1847) isolated the shell liquid by extracting with ether. He removed the anacardic acid by use of lead salts and tanins by treatment with water and finally concentrated the cardol by acidification of the alkaline liquid. Cardol thus obtained is not free from traces of lead and is considered not suitable for internal use. Krishnamurthy (1951) dissolved shell extract in petroleum ether and then treated it with aqueous alcoholic solution of sodium hydroxide. Later on, the cardol fraction was separated; but this method was not found suitable as this liquid mass had a strong tendency to emulsify during the initial stages of shaking the alkaline solution with ether and caused much inconvenience.

A method was devised for separation of cardol as follows:—

The shells were extracted for six hours with excess of 90 per cent alcohol which was drained off and the extraction was repeated with fresh

90 per cent alcohol by refluxing for 10 to 12 hours. The alcohol was then evaporated and the shell liquid recovered. This was then dissolved in minimum quantity of 90 per cent alcohol and treated with sodium bicarbonate solution; carbon dioxide was then passed into the solution till saturation. The separated cardol was extracted with ether, and then purified.

Cardol thus separated was found to be a thick brown liquid and irritant to the skin and mucous membrane like the whole shell extract. It was, therefore, put in hard gelatin capsules to be used on patients. A dose of 5 g. to 6 g. of the drug was used for adults who were indoor and outdoor patients of the hospital. Before administration of the drug, the patients were prepared as mentioned before. Out of 37 cases of *Ascaris* infection, 28 were cured by a single treatment. Out of 101 cases of hookworm infection 51 were absolutely cured and four more practically cured by a single treatment and nine more cases showed an egg reduction of 80 per cent or more. Of the uncured cases, five were given a second treatment and two were absolutely cured and one practically cured. From one case as many as 545 hookworms were recovered on the first day of treatment. The drug was well tolerated and appeared to be a safe and non-toxic remedy. Blood pressure recorded before one hour after the drug did not show any significant variation. The drug appeared to be as effective as the whole extract against *Hymenolepis nana*, five out of eight cases being cured with a single treatment. Of the 15 cases of *Enterobius vermicularis* infection treated, five could be followed and three were cured. From one case as many as 1,195 worms were recovered on the first day. Out of 29 cases of infection with *Trichuris trichiura* three were cured.

This fraction, therefore, appears to be a safe and non-toxic drug with a broad range of anthelmintic action and better than the whole extract of the shell and further study will be done with it.

Anacardic acid has also been extracted from the liquid left after the separation of cardol fraction.

9. Study of the modification by quinine of the hæmolytic response of erythrocytes to various hæmolytic agents under Dr. R. Singh Grewal at the Medical College, Nagpur.

Mer Birnbaum and Kligler (1940) using ox bile as hæmolytic agent showed that the erythrocytes from malarial subjects were hæmolysed more readily on administration of quinine. This suggests that the quinine administration renders the cells more sensitive to the action of hæmolytic agent. It was considered to be of interest to study this action of quinine in modifying the response of red blood cells to various hæmolytic agents both *in vitro* and *in vivo* in laboratory animals. A study of this nature would throw some light on the possible rôle of quinine in the production of intravascular hæmolysis as seen clinically in cases of black-water fever.

In this connection a photo-electric colorimeteric method had to be devised in order to assess the degree of hæmolysis produced by various hæmolytic agents.

The method is based on the principle of estimating the quantity of hæmoglobin in the supernatant of hæmolysed sample of blood and at the same time determining the total hæmoglobin content of that particular sample of blood. Taking the total hæmoglobin estimation as representing 100 per cent hæmolysis the degree of hæmolysis in a partially hæmolysed sample can be calculated in the following manner. If the total quantity of hæmoglobin of the whole blood is 15 g./100 c.c. and if the supernatant after hæmolysis gives a figure of 5 g. then the degree of hæmolysis is 33·3 per cent. The hæmoglobin estimation is done by converting it into acid hæmatin by adding 0·1 N hydrochloric acid and the intensity of the colour developed is read off from the photo-electric colorimeter. The quantity of hæmoglobin corresponding to a particular reading on the photo-electric colorimeter is determined by referring to a calibration curve. The calibration curve is prepared by plotting on semilogarithmic graph paper, the readings obtained on the photo-electric colorimeter against the solution containing known quantity of hæmoglobin converted into acid hæmatin. The solutions containing the known quantity of hæmoglobin are obtained from a blood sample in which the total hæmoglobin has been estimated by determining the amount of iron by photo-electric colorimeteric method of Wong. When readings are plotted as described above a linear relationship is obtained between the hæmoglobin concentration and photo-electric colorimeter reading. From this curve the quantity of hæmoglobin in a sample of blood can be read off directly when the photo-electric colorimeter reading of the sample is known.

To set up an experiment varying dilutions of the hæmolytic agent were used and 0·1 c.c. of heparinized blood was added to all the tubes. The tubes were kept at room temperature for 30 minutes and then centrifuged at slow speed for 15 minutes. The supernatant fluid contained hæmoglobin which was estimated in the form of acid hæmetin on the photo-electric colorimeter as described above.

By using this method the range of hæmolytic activity of quinine was determined for rabbit erythrocytes. Quinine is hæmolytic in dilutions up to 1 in 700 only.

Experiments were set up to find out whether quinine in dilution of 1 in 10,000 increased the degree of hæmolysis produced by saponin. Saponin obtained from two firms was used. Results obtained with saponin depuratum (Merck) in 4 experiments and saponin BDH in 5 experiments are summarized in Table I:—

TABLE.

Saponin dilution	hæmolysis with saponin (Merck), Per cent	hæmolysis with saponin (BDH), Per cent	hæmolysis with saponin + quinine 1 in 10,000, Per cent
1 - 5,000 ...	76	...	91
1 - 7,000 ...	28	...	45
1 - 10,000 ...	10	...	31
1 - 12,000 ...	*Nil*	...	5
1 - 15,000 ...	...	66	81
1 - 20,000 ...	...	56	73
1 - 30,000 ...	...	28	42
1 - 40,000 ...	...	13	22

Saponin (BDH) appeared to be a more powerful hæmolytic substance than Merck saponin depuratum.

It would be evident from the Table that quinine in $\frac{1}{15}$ times its hæmolytic concentration can increase the degree of hæmolysis produced by saponin.

10. Action of *Herpestis monniera*—Bacopa herb (Brahmi) on the nervous system under Dr. C. L. Malhotra, Professor of Pharmacology, Lady Hardinge Medical College, New Delhi.

The indigenous name ' Brahmi ' is confusing in the sense that it has been applied to two different species of plants, viz. *Herpestis monniera* and *Hydrocotyl asiatica*, in different regions in India. The present work is concerned with *Herpestis monniera.*

In Ayurvedic system of medicine, *Herpestis monniera* has been extensively used for various diverse nervous disorders such as insanity, epilepsy, hysteria etc., and has been claimed to be a 'nervine and cardiac tonic'. In the present studies, preliminary pharmacological studies of the dried alcoholic extract of *Herpestis monniera* have been carried out in frogs.

1. *Toxicity tests.*—Two hundred mg./100 g. body-weight given into the ventral lymph sac caused marked depression of respiration and death of all the frogs in 10 to 15 minutes. There was marked neurological deficit as shown by absence of righting reflex, loss of equilibrium and balancing, etc.

One hundred mg./100 g. body-weight doses had the same effect though the survival period varied from 1 to 2 hours. LD_{50} in case of frogs after injection into ventral lymph sac varied from 40 to 60 mg./100 g. body-weight.

2. *Nervous system.*—Graded doses of dried alcoholic extract from 15 mg./100 g. to 40 mg./100 g. body-weight injected into ventral lymph sac of the frog produced depression of respiration with varying degrees of neurological deficit. With 10 mg./100 g. body-weight of the extract given orally as well as into the lymph sac, there was, however, slight transient stimulation of respiration followed by a slight depression but there was no other neurological deficit. These studies suggest that the effect of the extract of *H. monniera* on nervous system of frogs is mainly depression though in small amounts it causes slight and transient stimulation of respiration.

3. *Reflex excitability.*—Doses varying from 10 mg./100 g. to 40 mg./100 g. body-weight injected as 1 per cent solutions depress reflex excitability in frogs.

4. *Nerve-muscle preparation of frogs.*—Solution of varying strengths, i.e. from 1 in 1,000 to 1 in 10,000 produced neuro-muscular block, the onset of complete block through nerve stimulation was earlier with higher concentrations than with lower ones. In each case, however, the muscle was responsive to direct muscle stimulation.

5. *Heart.*—(A) Continuous perfusion of the intact heart of frogs with dilutions varying from 1 in 1,000 to 1 in 10,000 gave the following results:—

(*i*) Initial increase in the heart rate.

(*ii*) Progressive increase in the strength of contraction of both auricles and ventricle. Soon the relaxation of the ventricle becomes imperfect. Later on the apex of the ventricle remains contracted during diastole. This state of contraction slowly spreads over the rest of the chamber until the ventricle stops in systole and the auricles unable to empty themselves come to stand still in the dilated position.

(*iii*) In certain instances, especially with higher concentrations, there was depression of the conduction of impulses from auricles to ventricle.

(B) With continuous perfusion of the isolated heart of frogs, the same effects were noted.

(C) After perfusion with 1 c.c. to 2 c.c. of different ilutions varying from 1 in 20,000 to 1 in 100,000, practically there was no effect on the intact heart. With 1 in 1,000 to 1 in 10,000 there was stimulation of the heart with increase in tone and rate, the intensity of action being proportional to the strength used. In each case the heart returned to normal activity These findings suggest that the extract has got direct stimulating effect on the cardiac muscle of frogs.

6. *Blood vessels.*—Continuous perfusion of the frog's blood vessels with different dilutions varying from 1 in 1,000 to 1 in 10,000 resulted in vasoconstriction which lasted as long as the perfusion was carried on. With amounts varying from 1 c.c. to 2 c.c. of different strengths there was again vasoconstriction, the intensity and duration of which varied with the strength used.

IV. ABSTRACTS FROM REPORTS OF I.C.M.R. RESEARCH FELLOWS.

1. Research on histoplasmosis by Dr. M. B. Ghosh at the Medical College, Calcutta.

Methods of study.—Between April 7, 1953 and April 6, 1955, 5,422 patients have been histoplasmin (1/100) and tuberculin tested (5 T.U. P.P.D.). The techniques were similar to those followed in Standard Mantoux method. Both fluoroscopic examination and skiagraphy of chest were taken in 3,050 cases, only fluoroscopic examination of chest was made in 5,072 cases and 350 cases had neither fluoroscopy nor x-ray examination of their chest. A detailed clinical history and physical examination findings were recorded. Cases which proved positive to the histoplasmin test were further investigated by examination of sputum of *H. capsulatum* by direct microscopy, staining by Gram's and Leishman's method and by culture in Sabourand's maltose agar medium at 37°C. Biopsy of tissues was made from enlarged glands or other biopsy materials wherever available and sternal marrow aspirations were made in few cases. Sputum were further examined for acid fast bacilli by Ziehl Neelsen's technique and by culture in Lowenstein-Zensen's media.

Results.—Out of these 5,422 cases, 567 cases could not be followed up and were excluded from the series so that the total number of patients investigated was 4,855. Of these, 3,105 were males, 1,750 females. All but 98 cases (mostly sailors) were never out of India.

Out of these 4,855 cases, 26 proved positive to the histoplasma antigen alone (0·53 per cent), 33 to both histoplasmin and tuberculin (0·67 per cent) 3,881 reacted to tuberculin alone (79·93 per cent) and 915 were non-reactors to either antigen (18·84 per cent).

The result is summarized in Table I.

TABLE I.

Prevalence of histoplasmin and tuberculin sensitivity among 4,855 *patients.*

Antigens	Number	Per cent
Histoplasmin only	26	0·53
Tuberculin only	3,881	79·93
Histoplasmin and tuberculin	33	0·67
Non-reactors	915	18·84
TOTALS ...	4,855	99·97

Of these 4,855 patients 190 were cases of pulmonary calcifications. Of the 190 cases of pulmonary calcifications, 14 proved positive to the histoplasmin antigen alone (7·36 per cent), 146 to tuberculin alone (76·84 per cent), 18 to both histoplasmin and tuberculin (9·47 per cent) and 12 were non-reactors to either antigen (6·31 per cent). *H. capsulatum* could not be isolated in any of these histoplasmin positive cases of pulmonary calcifications by different laboratory methods.

The prevalence of histopalsmin and tuberculin sensitivity among the 190 cases of pulmonary calcifications has been shown in Table II:—

TABLE II.

Prevalence of histoplasmin and tuberculin sensitivity among 190 *patients with pulmonary calcifications.*

Antigens	Reactors among 4,855 patients		With calcifications		Percentage reactors among 190 cases with calcifications
	Number	Per cent	Number	Per cent	
Histoplasmin only ...	26	0·53	14	53·84	7·36
Tuberculin only ...	3,881	79·93	146	3·76	76·84
Histoplasmin and tuberculin ...	33	0·67	18	54·54	9·47
Non-reactors ...	915	18·84	12	1·31	6·31
TOTALS ...	4,855	99·97	190	3·91	99·98

Correlation of the nature and distribution of pulmonary calcification to the positive histoplasmin test failed to show any typical pattern of pulmonary calcification amongst the positve reactors to the antigen.

The age and sex distribution to histoplasmin and tuberculin sensitivity is shown in Table III. The maximum incidence of histoplasmin positive reactors was noted between 3rd and 5th decade of life.

X-ray distribution of lesions among the 59 positive reactors to histoplasmin has been shown in Table IV. Of these 59 cases 26 reacted to histoplasmin alone ,and in this small group of 26 cases, 14 were cases of pulmonary calcifications, three cases of pulmonary infiltration, in one there was enlarged mediastinal shadow on the right side and in eight no definite lung infiltration or calcifications were detected.

The *x*-ray distribution of lesions among 59 positive reactors to histoplasmin is shown in Table IV.

The result of laboratory investigations for *Histoplasma capsulatum* is shown in Table V.

Attempts to identify *Histoplasma capsulatum* microscopically or by cultural methods failed and only negative results were obtained in this series.

TABLE III.

Prevalence of histoplasmin and tuberculin sensitivity among 4,855 patients by age and sex.

Age in years	Histo-plasmin		Tuberculin		Histo. & tuber.		Non-reactors		Totals
	M	F	M	F	M	F	M	F	
0–4 ...	...	...	36	47	...	...	91	114	288
5–9 ...	...	...	114	85	...	...	78	72	349
10–14 ...	2	...	140	99	...	...	52	41	334
15–19 ...	1	2	204	135	...	1	53	30	426
20–24 ...	4	2	301	180	2	2	49	17	557
25–29 ...	3	2	352	198	6	1	28	16	606
30–34 ...	4	...	339	179	4	1	18	8	553
35–39 ...	2	...	280	149	5	1	23	7	467
40–44 ...	4	...	255	126	6	...	19	6	416
45–49 ...	...	...	182	91	2	...	21	9	305
50–59 ...	...	...	151	48	1	...	31	13	244
60–69 ...	...	...	112	35	1	...	49	15	212
70–79 ...	...	...	34	9	...	...	46	9	98
All ages ...	20	6	2,500	1,381	27	6	558	357	4,855

TABLE IV.

X-ray distribution of lesions among the 59 *positive reactors to histoplasmin.*

Lesions	Histoplasmin only	Tuberculin and histoplasmin	Totals
Pulmonary calcification ...	14	13	27
Pulmonary infiltration ...	3	5	8
Enlarged mediastinal shadow ...	1	1	2
No lung infiltration ...	7	14	21
Others	1	...	1
TOTALS ...	26	33	59

TABLE V.

Result of laboratory investigations for Histoplasma capsulatum.

Methods	Material	Number	Results
Direct microscopy	Sputum:		
	(*i*) Wet preparations ...	177	Neg.
	(*ii*) Staining	296	
Culture	Sputum:	142	
	Skin biopsy material ...	11	
	Gland biopsy material ...	7	Neg.
	Laryngeal swab ...	2	
Histology	Skin	22	No *Histoplasma capsulatum* seen in tissues.
	Gland	14	
Aspiration ...	Bone marrow ...	7	Neg.
	Bronchoscopic ...	1	Neg.

2. Genital cytology in gynæcological and obstetrical cases by Dr. (Miss) K. Gupta at the S. N. Medical College, Agra.

Work on genital cytology in obstetrical and gynæcological cases was taken up with the following objects in view:

1. Study of vaginal smears during the various phases of the menstrual cycle in cases of clinically diagnosed primary and secondary sterility and a correlation with endometrium biopsy and chemical estimation of urinary pregnanediol.

2. Study of vaginal smears with a view to detect post-radiation changes in cell cytology in cases of carcinoma of the female genital tract.

3. Evaluation of vaginal cytology as a routine laboratory procedure for the diagnosis of early carcinoma of the genital tract.

4. Study of the changes in cytology during pregnancy, post-partum, menopause and inflammatory lesions of the genital tract.

During the period of this project 1,733 cases were studied as follows:

1. Sixty-two cases of primary and secondary sterility were studied. It was found that fairly accurate assumption of ovulation can be obtained from the study of smears in the second half of the menstrual cycle. The observations are well correlated with the findings of endometrial biopsy and chemical estimation of pregnanediol.

2, Fifty-three post-radiation cases were studied. The cytological findings reveal characteristic changes both in normal and malignant cells. The changes considered specific of radiation are bizarre shapes, cytoplasmic vacuoles, endophagocytosis associated with a marked increase in the sizes of the cell, including both the nucleus and the cytoplasm. It is felt that it is of value in early diagnosis of recurrent carcinoma.

3. Cytological diagnostic criteria for detection of cancer cells has been studied. 1,618 cases were screened. These comprised women above 30 years of age with or without gynæcological symptoms. As a result of this study it can safely be concluded that cytological studies afford an important screening test in cervical carcinoma.

4. Three hundred and forty-one cases were studied under the entity of cervical erosions. A marked exfoliation of basal and parabasal cells was usually seen. They occasionally present anisocytosis and anisonucleosis, but each cell has a distinct cytoplasmic border. Macronucleolus was never observed. It is apparent from this study that a single criterion is not sufficient to establish conclusively the diagnosis of malignancy.

Two hundred and ninety-two cases were studied under menopause. A marked exfoliation of basal and parabasal cells and a paucity of superficial squamous cells were the striking findings.

Eighty-eight cases studied under pregnancy show that only about ten per cent cases exfoliate navicular cells which are described as characteristic of pregnancy by Papanicolaou.

3. The etiology of *Thromboangitis oblitirans* by Dr. G. K. Sharma at the K. G. Medical College, Lucknow.

CLINICAL WORK.

A total of 25 cases of Burger's disease were studied during the fellowship period.

The following important points were noted in the history and examination of the patients:—

It was found that at the time of admission all patients had local pain, 96 per cent had rest pain, duration varying from one month to one year. Trophic changes were found in 72 per cent of cases.

All the patients were smokers and out of these 68 per cent were heavy smokers consuming more than ten cig./day. Forty per cent of the cases gave history of indulgence in bhang, ganja and charas. Thrombophabitis was found in 36 per cent of cases. Exposure to cold and trauma was found to be the aggravating cause in 16 per cent of cases. The various investigations, such as r.b.c., w.b.c., Hb. per cent, difference count, e.s.r., p.c.v., bleeding and coagulation time, W. R. blood culture, venous pressure and blood pressure, did not show any abnormality either in pre-operative or postoperative periods.

Treatment.—The medical treatment by prescol, vitamine E, I.V. saline and stilbesterol hormone did not produce any change in local or general condition of patient. Amputation was found to be the treatment of choice in two cases and sympathectomy was performed in all the other cases who were willing to undergo operative treatment. It produced good results in 70 per cent of cases. In these patients gangrene healed spontaneously local pain and rest pain was relieved by operation, but there was no effect on claudication time.

In four cases sympathectomy precipitated massive gangere and limb has to be amputated higher up in leg or thigh to save the patient.

Histology.—Histological picture of the six amputated limbs was studied and the observations recorded were identical to those recorded by other workers except that there were no prulent foci with Giant cell infiltratius as reported by Burger's. The disease process was found in various stages of its development in these cases from earliest stage of cellular infiltration, recent thrombosis to organization and recanalization of thrombus.

EXPERIMENTAL WORK.

A. Tobacco extract was prepared by soaking tobacco leaves in water for 48 hours.

B. The extract was quantitatively analysed for the presence of alkaloids other than nicotine. It was found that 1 c.c. of extract contained 1·439 mg. of alkaloids.

C. M.l.d. was determined by biological methods for a rat weighing 100 g. and was found to be 0·075 c.c. when given intraperitoneally.

D. Toxicity tolerance of rats was studied for a period of four weeks, the starting dose was less than I.m.l.d. and this was gradually increased to 0·12 c.c. intraperitoneally, a further increase showed 30 per cent mortality.

In this experiment extract was accidentally injected reteroperitoneally in rat No. 5 of batch I. This rat developed a lump in reteroperitoneal region envalving kidney, ureter and testis. Histological study revealed a picture of fibrosis and local necrosis with intense cellular infiltration, there was no damage to kidney tissue.

1. *Experimental production of Burger's disease in rats by intraperitoneal injections of tobacco extract.*

In this experiment, ten male, ten female and five ovarectomized female rats were kept under observation for 20 weeks. They received an initial dose of ·002 c.c./g. which was gradually increased to ·0065 c.c./g. In all 70 injections were given on alternative days.

The rats were sacrificed after 20 weeks and histological study of arteries done. Following changes were found in three male rats, i.e. rat 2, batch I and rat 3 and 4 of batch II.

Fibroblastic proliferation in adventatia.
Reduplication of internal elastic lamina.
Thrombus in the lumen of the artery with damage to intima at places.

2. *Experimental reproduction of Burger's disease in rats by embedding the thrombosed artery and veins near the femoral arteries of rats.*

The diseased arteries were taken from three cases of Burger's disease and were embedded in 15 male rats. During collection of material and embedding strict asepsis was maintained. The embedding was done on the right side only. After eight weeks of observation all the rats were sacrificed and histological picture of arteries studied. It was seen that there was intense fibrosis in muscles and periarterial tissue. There were no changes in the arterial tissue itself.

3. *Experimental production of Burger's disease in rats by combined injections of male sex hormone and tobacco extract.*

In this experiment ten male and ten female rats were kept under observation. They were given intraperitoneal injections of tobacco extract and intramuscular injections of testosterone propionate in oil, bi-weekly, over a period of 20 weeks.

A total of 108 injections of tobacco extract were given. Initial and final doses being ·001 c.c./g. and ·0025 c.c./g., respectively. Dose of testosterone was 65 mg. per rat and in all 38 injections of testosterone were given.

Results on post-mortem and histological examination—no changes were found in the arterial tree.

4. *Experimental reproduction of Burger's disease by producing vitamin E deficiency in rats and intraperitoneal tobacco extract.*

Ten male and ten female rats received synthetic diet from which vitamin E had been excluded. The diet regime was started eight weeks before starting the injection was continued after works, for another 18 weeks rats were given 100 intraperitoneal injections of tobacco extract over a period of 18 weeks inteal and final doses given were ·001 c.c./g. and ·0025 c.c./g. On post-mortem and histological examination after 18 weeks no change was found in the arterial tissue.

5. *Experimental production of Burger's disease by exposing rats to tobacco smoke in a special smoke chamber.*

The smoke chamber was devised and constructed locally. Twenty male rats were kept under observation. A batch of five rats was exposed to smoke of two cig./day over a period of 28 weeks. It was found that rat No. 4 of Batch IV developed gangrene tip of tail in the 26th week and this gradually spread up to 2½″ proximally. Histological appearance of the artery showed increased periarterial fibrosis and cellular reaction with normal musculature in the media. The elastic lamina is reduplicated. There is thrombus in the lumber which is of recent origin and is not organized.

From the above work following important conclusions were drawn:—

Tobacco smoking and its degree were directly related to severity of disease and in course. Indulgence in *ganja* and *charas* increased the severity of the manifestations. Attacks of thrombophilibitis and their number had no effect on the course of the disease although it preceded the disease in many cases, it is suggestive of a susceptibility of arterial trace of patient to the harmful agents.

Better results can be obtained after sympathectomy if patients are selected after consideration of pre-operative trophic changes their duration and course along with arteriographic and oscillometeric finding of limb.

Experimentally, it was proved that under suitable conditions, and if given for a sufficient length of time, tobacco can produce gangrene in rats and arterial pathology similar to that seen in Burger's disease.

4. Cardiac function in anæmia (A clinical and experimental study) by Dr. M. C. Khorwal at the K. G. Medical College, Lucknow.

The work done consisted of three parts:—

1. Clinical observations in cases of anæmia.
2. Investigations in the same cases.
3. Experimental observations on chronic anæmia in dogs.

PART I—CLINICAL.

Material.—Eighty-seven cases of anæmia who had a total r.b.c. count below 3 mill./cmm. and Hb. below 60 per cent, but had no evidence of any other organic heart disease were studied. There were 52 males and 30 females. Their ages ranged from 14 to 66 years with an average of 32·8 years. The illness was of gradual on set in all cases. The duration of illness ranged from one month to five years. The period of observation in the hospital ranged from one to three months.

In each case, on admission, a thorough history was taken regarding the presence, severity and duration of the cardiac symptoms, such as dyspnœa, palpitation, precordial pain and œdema. Complete blood examination was done on admission which included total r.b.c. count, hæmoglobin percentage, m.c.v., p.c.v., m.c.h., m.c.h.c., total w.b.c., differential w.b.c., platelet and reticulocyte counts. Total r.b.c. counts, hæmoglobin percentage and absolute values were repeated at weekly intervals and the effect of improvement in anæmia on different symptoms and signs were noted. Other blood examinations were repeated only when necessary. Pulse rate, blood pressure and complete physical examination of the heart, were done on admission and then observed at weekly intervals (on the day of blood count).

Venous pressure, circulation time, vital capacity and breath holding capacity were measured on admission and then repeated at two weekly intervals (on the day of blood count). Total blood volume estimation was done on admission in 22 uncomplicated cases (without congestive heart failure) of anæmia. The size of the heart was clinically estimated as a routine and confirmed in each case by screen examination before treatment. In some cases teleradiograms were taken. Those findings were then correlated with the blood counts done on the same day. In 75 cases, an electrocardiogram was taken on admission and if it showed some electrocardiographic abnormality another E.K.G. was taken after treatment. Three standard leads (L1, L2, L3), three unipolar limb leads (aVR, aVL and aVF) and six unipolar pericardial leads of the 'V' series (V1-V6) were taken each time.

In addition to bed rest, appropriate treatment of anæmia was done according to its type and ætiology. These observations continued in every case till the hæmoglobin level reached 80 per cent. But in six cases this level of hæmoglobin could not be attained in spite of the most careful and prolonged treatment.

Symptoms :

(*i*) *Lassitude.*—Varying degree of lassitude specially after exertion was present in all cases.

(*ii*) *Dyspnœa.*—Mild dyspnœa on exertion was noted in 52 cases. Marked dyspnœa (including orthopnœa) was present in ten cases, out of which seven cases had congestive heart failure.

(*iii*) *Palpitation.*—Mild palpitation on exertion was felt in 42 cases. Severe palpitation even at rest was felt in six cases.

(*iv*) *Precordial pain.*—Mild precordial pain after exertion, simulating angina pectoris was felt in six cases. Severe precordial pain after exertion was felt in one case.

All the above symptoms gradually disappeared in all cases, with improvement in blood condition.

Signs.—Mild œdema was present in 34 cases, moderate œdema was present in 15 cases and general anasarca was noted in ten cases.

(*i*) Eight patients of general anasarca had congestive heart failure. Total serum proteins ranged from 3·7 to 6·5 grammes per cent, serum albumin ranged from 2 to 5·5 g. per cent and serum globulin ranged from 1·2 to 3·2 g. per cent. In most of these cases albumin : globulin ratio was reversed. It was noted that in none of these cases the albumin was below the critical level of 2 g. per cent which is said to be responsible for causing the œdema. It can, therefore, be concluded that hypoproteinæmia alone in these cases is not responsible for the causation of œdema.

Degree of anæmia had no correlation with the severity of œdema. Increased capillary permeability due to anoxia and recurrent transient elevations of venous pressure have been suggested to be responsible for the precipitation of œdema in these cases.

(*ii*) *Pulse.*—Tachycardia (above 90/mt.) was noted in 22 cases. Water-hammer character of pulse was found in seven cases. These abnormalities disappeared after treatment.

(*iii*) *Cardiac sounds.*—In 19 cases both the cardiac sounds were found to be feeble in all the areas and in all of them they became normal after treatment. They are considered to be due to myocardial anoxæmia due to anæmia.

In 30 cases the second pulmonary sound was accentuated. It is considered to be due to increased pulmonary circulation. It became normal after treatment.

(*iv*) *Cardiac murmurs.*—Following murmurs were noted in 76 cases. In 11 cases no cardiac murmurs were found. These murmurs had no conduction.

Degree, duration or type of anæmia or any particular ætiological factor of anæmia was not found to be directly correlated with the presence of cardiac murmurs. In majority of the cases cardiac murmurs decreased or disappeared after improvement in blood condition.

Increased velocity of blood was noted in all these cases and so increased circulation rate and decreased viscosity of blood in these cases are considered to be mainly responsible for the causation of cardiac murmurs.

Murmurs present	Before treatment	AFTER TREATMENT		No change
		Disappeared	Decreased	
Mitral area only (soft syst.)	7 cases	3 cases	4 cases	
Pulm. area only (soft syst.)	5 ,,	1 cases	2 ,,	2 cases
Both mitral and pulm. area (Soft syst.)	33 ,,	14 cases	14 ,,	3 ,, 2 expired
(Harsh syst.) ...	5 ,,	...	3 ,,	2 cases
All over the precardium (Soft syst.)	26 ,,	10 cases	11 ,,	3 cases 2 expired

(*v*) *Cardiac enlargement.*—The generalized cardiac enlargement was present in 24 cases. Mitralized type of enlargement was noted in four cases. After treatment, the cardiac enlargement completely disappeared in ten cases, and in 16 cases only partial reduction in size took place, probably indicating permanent cardiac damage in the latter cases. Ten of these cases developed congestive heart failure. Duration or severity of anæmia alone were not directly correlated with the cardiac enlargement. Physical exertion during the anæmic stage is more responsible for the causation of cardiac enlargement.

(*vi*) *Congestive heart failure.*—It was present in ten cases. After treatment eight cases were completely relieved of this complication. Two cases expired. Cardiac enlargement together with severe degree of anæmia was the most important pre-disposing factor for congestive heart failure. Physical exertion is considered to be the precipitating factor for congestive heart failure, in these cases. The management of these cases is difficult and prognosis is poor. Mortality 20%.

PART II—INVESTIGATIONS.

I. *Blood.*		*Cases*
(*i*) *Type of anæmia:*	Microcytic anæmia ...	42
	Normocytic anæmia ...	25
	Macrocytic anæmia ...	20
(*ii*) *Degree of anæmia:*	Hæmoglobin below 20 per cent	24
	,, 21 to 40 per cent	42
	,, 41 to 60 per cent	21

(*iii*) Pancytopenia was present in four cases of splenic anæmia.

(*iv*) General blood picture examination showed varying degree of anisocytosis and poikilocytosis in most cases.

II. *Hæmodynamics:*

(*i*) *Blood pressure.*—Average rise of systolic and diastolic pressures, after treatment was found to be 7 mm. and 12 mm. of Hg. respectively.

(*ii*) *Circulation times.*—Arm to lung time (by ether method) and arm to tongue time (by saccharine method) showed increased velocity of blood in all cases of anæmia. The increase in rate of circulation was directly proportional to the severity of anæmia.

(*iii*) *Venous pressure.*—In all the uncomplicated cases (without congestive heart failure) the venous pressure was found to be more than normal, with an average of 112·2 mm. of normal saline. After treatment it decreased in all cases to an average of 88·4 mm. of normal saline.

(*iv*) *Blood volume.*—It was estimated in 22 cases only. The absolute and relative blood volumes were found to be increased in three out of four cases of splenic anæmia and in only one out of the 18 cases of other types of anæmia. In the remaining 18 cases, they were found to be mostly below normal. Absolute plasma volume was found to be increased in majority of the cases and was normal in only a few of them. Relative plasma volume (per Kgm. % body weight) was found to be increased in every case of anæmia.

III. *Electrocardiograms.*—Different E.C.G. changes, e.g., tachycardia, wide P wave, prolonged P-R interval, low voltage of QRS complex, depressed ST segment, flattening or inversion of T wave, prolonged Q-T interval, acute coronary insufficiency, were noted in 67 cases of anæmia. In majority of the cases the E.C.G. changes diasppeared after treatment. But in a few of the cases the E.C.G. changes persisted in spite of the cure of anæmia, thereby indicating the presence of permanent cardiac damage in them. Severity of anæmia or cardiac enlargement had no direct correlation with the presence or absence of E.C.G. changes.

IV. *Respiratory tests.*—Both breath-holding capacity and vital capacity increased after improvement in blood condition. Vital capacity was found to be a better index of judging the improvement in the general condition of the patient.

V. *Gastric analysis, urine and stool examinations.*—They were done to help in the proper diagnosis and management of these cases.

VI. *Ætiological diagnosis.*	*Cases.*		*Cases.*
Ankylostomiasis ...	33	Pernicious anæmia ...	3
Nutritional ...	9	Amœbiasis ...	3
Colitis	7	Abdominal tuberculosis	2
Diarrhœa ...	5	Hepatitis ...	2
Bleeding piles ...	4	Ass. with pregnancy ...	2
Unknown ætiology ...	3	Ascariasis ...	1
Splenic anæmia ...	4	Menorrhagia ...	1
Cirrhosis of liver ...	3	Peptic ulcer ...	1
Chronic malaria ...	3	Nephritis ...	1

PART III—EXPERIMENTAL.

Following hæmodynamic changes were studied in dogs during chronic anæmia:—

(*i*) Circulation time—(by Papaverine method).
(*ii*) Blood volume—(by Evans blue dye).

Standardization of techniques.—The techniques of doing circulation time and blood volume were standardized by doing these investigations in

15 healthy dogs and finding out the normal range of these investigations. The results obtained showed the wide range of normal circulation time and normal blood volume in healthy dogs.

Chronic anæmia in dogs.—Two healthy dogs were selected and then by bleeding them (50 c.c. bi-weekly), chronic anæmia was produced in about six to seven weeks (Hb. level was reduced to about 25 per cent, i.e., 3·2 g. per cent). Blood volume and circulation time were estimated on starting experiment and then repeated at weekly intervals till after seven days of the last bleeding. The initial and the final values were then compared to find out the changes resulting from anæmia. Following changes were found during chronic anæmia:—

(*i*) Increased circulation rate in all the animals.
(*ii*) Increased, absolute and relative plasma volumes in all.
(*iii*) Decreased absolute blood volume in all.
(*iv*) Relative blood volume was found to be variable.

These findings are in conformity with the observations made in patients of anæmia.

DISCUSSION.

The different cardiac symptoms and signs found in cases of anæmia and their pathogenesis have been described in the previous pages. Most of these symptoms and signs appear when the hæmoglobin level is below 40-50 per cent. It is of vital importance to carefully differentiate these cases of anæmia with cardiac symptoms and signs from other cases of organic heart disease, because we can mostly cure the former but not the latter. Lassitude, dyspnœa, precordial pain and palpitation were relieved in all cases of anæmia after improvement in blood condition.

Cardiac murmurs disappeared or decreased in majority of cases after treatment but they were found to have no direct co-relation with the severity or duration of anæmia or cardiac enlargement alone. So it is thought that decreased viscosity of blood and increased circulation rate may be responsible for these murmurs. It is possible that more than one factor may be responsible for the causation of cardiac murmur in a particular case.

It is difficult to say whether myocardial anoxæmia or the disturbed dynamic circulation (increased venous pressure, increased velocity of blood and raised cardiac output), plays more important rôle in the production of cardiac enlargement, because both of them are present in all cases of anæmia. The cardiac enlargement was found to have no direct co-relation with the severity or the duration of anæmia alone, in these cases, and therefore, the rôle of myocardial anoxæmia, as the primary cause of cardiac enlargement does not seem to be significant. It has been concluded from the present observations that physical exertion during the anæmic state is more responsible for the causation of cardiac enlargement. Physical exertion during anæmia leads to excessive hæmodynamic changes which cause severe increase of the diastolic volume of the heart resulting in its dilation, hypertrophy and ultimate enlargement.

It was noted that in some patients, a few of the cardiac findings e.g. cardiac enlargement and E.C.G. changes, persisted even after the cure of anæmia. This indicated the irreversible cardiac damage done by chronic anoxæmia, in these cases.

Increased absolute plasma volume was found in majority of the patients. Increased relative plasma volume was found in all patients and animals during anæmia. These changes may be compensatory for the decreased cell volume of the anaemic blood and help in keeping the circulatory blood volume as near normal as possible. Absolute and relative blood volume were found to be decreased or normal in majority of the cases and were increased in only a few of them. It was interesting to note that unlike in other types anæmia, the average absolute and relative blood volumes in three out of the four cases of splenic anæmia were found to be very high.

To compensate for the low oxygen carrying capacity of blood in anæmia certain circulatory changes take place e.g. tachycardia, increased velocity of blood and raised cardiac output. In the present series tachycardia (above 90/mt.) was present in 22 cases only. Increased circulation rate was found in all patients of anæmia and in both the dogs during chronic anæmia. Increased venous pressure was found in all patients of anæmia. From these facts it is confirmed that the increased velocity of blood resulting in increased venous return is mainly responsible for the increase of the cardiac output reported in cases of anæmia.

The different cardiovascular changes occurring in anæmia may be summarized as following:—

In anæmia the efficiency of myocardium is decreased due to anoxæmia and so the cardiac reserve is slightly decreased. Simultaneously certain hæmodynamic changes occur in the body to compensate for the low oxygen carrying capacity of the blood. (Tachycardia, increased circulation rate and increased venous return are the most important of these changes). These changes result in increased cardiac output, which puts an additional strain on the anoxæmic myocardiam and further decreases the patients cardiac reserve. Physical exertion greatly accelerates all these homodynamic changes which if severe result in excessive increase of the diastolic volume of the heart and cause its dilatation a hypertrophy and ultimate enlargement. This still further decreases the cardiac reserve. In a case of severe anæmia the presence of cardiac enlargement indicates that his heart has been sufficiently strained. Cardiac enlargement together with severe degree of anæmia is the most important pre-disposing factor in the causation of congestive heart failure. And so at this stage, if the patient of severe anæmia remains untreated and continues to strain his already anoxaemic myocardium by doing physical exertion beyond his extremely limited cardiac reserve, the congestive heart failure gets precipitated. The management of such cases is difficult and their prognosis is poor. (Mortality is 20 per cent). Therefore, the cases of anæmia should not be allowed to be neglected to the stage, when congestive heart failure can easily occur. Early diagnosis of these cases can be easily done if in every case of anæmia it is kept in mind that anæmia itself can be responsible for the several cardiac manifestations present in a particular case. Such cases should always be treated early to avoid permanent cardiac damage due to chronic anoxæmia. Physical rest and appropriate anti-anæmic theraphy should be instituted in these cases as early as possible.

CONCLUSIONS.

(i) Several cardiac symptoms and signs e.g. dyspnœa, palpitation, precordial pain, œdema, changes in heart sounds, cardiac murmurs, cardiac enlargement, congestive heart failure, and E.C.G. changes were seen in cases of moderate and severe degree of anæmia.

(ii) They disappeared in the majority of the cases after treatment, but in some patients a few of these findings e.g. cardiac enlargement and E.C.G. changes persisted in spite of the improvement in anæmia. This is considered to be an indication of the permanent cardiac damage caused by chronic myocordial anoxæmia due to anæmia.

(iii) Cardiac enlargement, Cardiac murmurs, E.C.G. changes and congestive heart failure were found to have no direct correlation with either the severity or the type or the duration of anæmia alone, because these findings were absent in several cases of severe anæmia of quite a long duration.

(iv) Physical exertion has an important rôle in the causation of cardiac enlargement and congestive heart failure.

(v) Cases of severe anæmia with enlarged heart are most liable to develop congestive hearts failure if they are not treated early and continue to do physical exertion and strain the heart beyond its limited reserve. Cardiac enlargement associated with severe degree of anæmia, is an important pre-disposing factor in the causation of congestive heart failure.

(vi) The management of cases of anæmia with congestive heart-failure is difficult and their prognosis is poor (mortality 20 per cent).

(vii) To compensate for the low oxygen carrying capacity of blood in anæmia certain circulatory changes were found to take place. (a) Tachycardia was noted in 22 cases only, at normal body temperature. Tachycardia seen in presence of pyrexia cannot be considered in this connection. (b) Increased circulation rate was found in all cases of anæmia. (c) Increased venous pressure was found in all cases of anæmia. From these facts it is confirmed that the increased velocity of blood resulting in increased venous return is mainly responsible for the increase of the cardiac output reported in cases of anæmia.

(viii) Increased relative plasma volume (per kgm. of body weight) was found in all patients of anæmia. In majority of the cases, the absolute plasma volume was found to be increased. Both, absolute and relative blood volumes were found to be decreased or normal in majority of the cases and were increased in only a few of them. The average absolute and relative blood volumes in three out of the four cases of splenic anæmia were found to be very high.

Increased absolute plasma volume and relative palsma volume, as found in majority of these cases, may be compensatory for the decreased cell volume of the anæmic blood and help in keeping the circulatory blood volume as near normal as possible.

(ix) Pathogenesis of the different cardiac symptoms ad signs found in anæmia has been discussed in the report.

(x) Experimental work on chronic anæmia in dogs showed the following hæmodynamic changes:—(during anæmia)

(a) Increased circulation rate in all the animals.
(b) Increased, absolute and relative plasma volumes in all.
(c) Decreased absolute blood volume in all.
(d) Relative blood volume was found to be variable.

These experimental results are in confirmity with the clinical observation that increased velocity of blood is the most important hæmodynamic change in anæmia and is responsible for the increased cardiac output reported in this condition.

5. Biological races of anopheline species with special reference to the salivary gland chromosomes and their bearing on speciation by Shri N. Rishikesh at the University College, Trivandrum.

A study of the detailed larval chætotaxy of three of the four vector anopheline species of the State, viz., *culicifacies*, *varuna* and *jeyporiensis* var. *candidiensis* was carried out with a view to disclose whether minor variations exist between local populations. In *culicifacies* variations of possible significance are evident in hair No. 2 of the metathorax and hair No. 13 on the prothorax and on the fifth abdominal segment. *Jeyporiensis*-var. *candidiensis* exhibit small differences in the number of branches of the inner shoulder hair on the prothorax and hair No. 4 on abdominal segments 5 and 6. No significant variations have been noticed in *varuna*.

The larval salivary gland chromosomes of *A. barbirostris* and *A. hyrcanus* var. *nigerrimus* have been studied in detail. This study has confirmed Dr. Frizzi's findings in Italy that with due attention to the culture of the larvæ, satisfactory salivary chromosome preparations of the mosquito can be procured which would enable their mapping. Separate races in a composite species may be easily recognized on the basis of such salivary chromosome maps. The salivary chromosomes of four other anopheline species, including the vector species *culicifacies* and *varuna*, have also been tentatively studied. A limitation in the way of a detailed study and mapping of these chromosomes, however, is the impracticability of colonizing the eurygamous anopheline species in the laboratory without which a continuous and plentiful supply of genetically homogeneous material could not be obtained.

6. Studies on the incidence, nature and types of Coxsackie virus infections in Bombay City by Miss S. M. Pathak at the Seth G. S. Medical College, Bombay.

The ubiquitous nature of Coxsackie viruses is wellknown and numerous isolations of these viruses have been made in many parts of the world from sewage, flies and from fæces of patients with clinical diagnosis of paralytic and non-paralytic poliomyelitis, epidemic pleurodynia, encephalitis, herpangina and brief febrile maladies without distinctive features (*Ann. Rev. Microbiol.*, **5,** pp. 304-332, 1951). With a view to investigating Coxsackie virus infections in Bombay City, the following materials were collected: (*a*) (*i*) Stool and throat swab samples for three consecutive days and (*ii*) whenever possible both ' acute ' and convalescent phase blood samples from patients suffering from poliomyelitis, pleurodynia, encephalitis, etc., (*b*) autopsied brain suspensions from suspected encephalitis cases admitted at the King Edward VII Memorial Hospital, and (*c*) fruit flies and various species of flies collected in different localities in Bombay. A total collection of material from 202 cases was made for the investigation of Coxsackie viruses.

Out of the total collection, processed fæcal samples from 70 poliomyelitis, 19 encephalitis, 4 pleurodynia and 4 ascending myelitis cases and 15 flies suspensions were put to test by inoculation into newborn mice (in each case a litter of six to eight new-born mice was inoculated subcutaneously, each mouse receiving 0·02 c.c. of the inoculum. In the suspected cases of encephalitis and in case of flies suspension each new-born mouse received 0·02 c.c. of the inoculum subcutaneously and 0·01 c.c. intracerebrally. To each inoculum a mixture of penicillin and streptomycin in the concentration of 120 units and 0·5 mg. per c.c., respectively, was added.

A single mouse passage and histological examination revealed the presence of Coxsackie viruses from the fæces of 21 poliomyelitis, 2 encephalitis, 1 pleurodynia and 1 ascending myelitis case. Two more positive isolations were made from fruit flies samples. One of these strains was from fruit flies collected in our research laboratory and the other one was from those collected at Matunga. Majority of dositive isolations were made from patients below four years of age, and who were admitted to hospital during the months of May to October of the year.

Cross neutralization tests on 12 of the local strains, in new-born mice, indicated the endemicity of infection due to at least six antigenically different strains, viz., Dalldorf's types 1, 3, 4, 10, 12 and 14. Seven out of 12 strains tested, belonged to type 4 indicating thereby the heavily endemic nature of infection by this strain in Bombay.

All attempts made to adapt to chick embryos, the locally isolated Coxsackie virus strains, met with failure. Attempts were also pursued to grow these local strains in *rhesus* monkey kidney cell tissue culture in stationary tubes. Two blind passages of these strains failed to produce any cytopathogenic changes in the infected explants.

Intracerebral monkey inoculation (kindly carried out by Indian Council of Medical Research Polio Unit at Grant Medical College, Bombay) of 35 fæcal samples revealed the presence of poliomyelitis virus in seven cases. In two of these cases there was simultaneous presence of Coxsackie virus as found out by new-born mice inoculations.

V. INDIAN JOURNAL OF MEDICAL RESEARCH AND INDIAN MEDICAL RESEARCH MEMOIRS.

1. (*a*) *Indian Journal of Medical Research, Volume XLII*, 1954.—January number contained 150 pages and four plates, April number 156 pages and three plates, July number 182 pages and eight plates, and October number 208 pages and four plates.

(*b*) *Memoir*.—One Memoir (*Memoir* No. 38—" Refraction and Body-growth " by G. S. Pendse) was published with Volume XLII.

(*c*) *Supplementary number*.—One supplementary number entitled " Review on Toxæmia of Pregnancy " by R. K. K. Tampan and Indira Ramamurthy was also published with Volume XLII.

2. (*a*) *Indian Journal of Medical Research, Volume XLIII*, 1955.—The January number contained 170 pages and 11 plates, April number 198 pages and 15 plates, July number 190 pages and 18 plates and the October number is expected to contain 230 pages and 20 plates.

(*b*) *Supplementary number*.—One supplementary number is expected to be published with Volume XLIII.

VI. INDIAN JOURNAL OF MALARIOLOGY.

The September and the December 1954 issues of Vol. VIII and the March and the June 1955 issues of Vol. IX were published during the year under report.

Besides contributors in India, the Journal continues to enjoy the confidence of research workers in the foreign countries as well. Of the 48 papers published in Vol. VIII, 17 were submitted by workers from Algeria, Belgian Congo, Belgium, France, Pakistan, Rome, U.K., and U.S.A.

Requests from institutions in India and abroad are received regularly for inclusion in the Exchange Mailing List of the Journal. Where the literature published by the parties is considered sufficiently useful, such requests are invariably entertained. The following table shows the distribution of the Journal:—

Subscribers—		
Foreign countries	70	
In India	98	168
Exchange mailing list		114
Free mailing list		112
Voucher copies to advertisers, specimens, propaganda, etc.		25
The Malaria Institute of India		61
Balance held by the publishers for sale		120
		600

The annual subscription of the Journal is Rs. 12/- per volume consisting of four numbers. The cost of a single copy is Rs. 5/- per number.

VII. MICROFILM AND PHOTOCOPY SERVICE UNITS.

(a) At the Central Research Institute, Kasauli.

During the period under review 53 requests for photostat copies and microfilms were complied with. Sixteen requests could not be entertained on account of the non-availability of the relevant journals.

Nine hundred and eighteen pages of photostat copies and 246 pages of microfilms were supplied to medical institutions and research workers.

The following table gives the photostat and microfilm work done during the period October, 1953 to September, 1954 and October, 1954 to September, 1955.

		1953–54	1954–55
Photostat copies	...	798 pages	918 pages
Microfilms	...	754 ,,	246 ,,

There were 13 requests for laboratory photographs and photomicrographs. During the period October, 1954 to September, 1955, 60 laboratory photographs and 32 photomicrographs were supplied as compared to only four supplied during October, 1953 to September, 1954.

There has been appreciable decrease in the supply of microfilms this year. Main indentors for them were INSDOC, New Delhi. Supply of photostat copies has, however, slightly increased.

(b) At the Indian Cancer Research Centre, Tata Memorial Hospital, Bombay.

During the period under review 79 requests for microfilms and photocopies from research institutions and research workers were complied with. Four hundred and eighty-four pages of photocopies and 3,594 pages of microfilms were supplied to medical institutions and research workers.

		1953–54	1954–55
Photocopies	...	393	484
Microfilms...	...	2,839	3,594

The rates for the supply of microfilm and photostat copies are:—

Microfilm:		Rate Rs. as. p.
For the first ten pages or less	... ...	3 0 0
For the subsequent ten pages or any portion thereof	...	1 8 0

Photostat:		Per page
Size up to 11″× 6″	... ...	0 8 0
,, ,, ,, 17″×11″	... ...	0 12 0
,, ,, ,, 23″×17″	... ...	1 14 0

VIII. PAPERS PUBLISHED BY WORKERS OF THE INDIAN COUNCIL OF MEDICAL RESEARCH DURING THE YEAR 1955.

A. *In the Indian Journal of Medical Research.*

1. AHUJA, M. L., and SURI, J. C.—Antirabies Treatment (Vol. 43, No. 3).
2. ANAND, B. K.—Somnolence caused by Destructive Lesions in the Hypothalamus in Cat (Vol. 43, No. 2).
3. ANAND, B. K., and DUA, S.—Blood Sugar Changes Induced by Electrical Stimulation of the Hypothalamus in Cat (Vol. 43, No. 1).
4. ANAND, B. K., and DUA, S.—Feeding Responses Induced by Electrical Stimulation of the Hypothalamus in Cat (Vol. 43, No. 1).
5. ARORA, R. B.—The Antiveratrinic Action of Cinchona Alkaloids, Cupreines and Synthetic Antimalarial Drugs (Vol. 43, No. 2).
6. ARORA, R. B., and DAS, P. K.—Barbiturates on the Automaticity of Sino-auricular and Auriculo-ventricular Nodes (Vol. 43, No. 4).
7. ARORA, R. B., SHARMA, V. N., and MADAN, B. R.—Chloroquine in Auricular Fibrillation (Vol. 43, No. 4).
8. BALASUBRAMANIAN, S. C., LILY, G., MANI, G. S., and BASU, K. P.—Nutritive Value of the Proteins of Milk and Some Indigenous Milk Products (Vol. 43, No. 2).
9. BANERJEE, S., CHATTERJEE, R., and BELAVADY, B.—Glucose Tolerance Test in Health and in Typhoid Fever (Vol. 43, No. 2).
10. BANERJEE, S., and LAHIRI, S.—Studies on the Biosynthesis of Nicotinic Acid in Germinating Pulses (Vol. 43, No. 2).
11. CHAKRAVARTI, N. K., CHAUDHURI, R. N., and WERNER, G.—Observations on Vascular Changes Produced by Argemone Alkaloids (Vol. 43, No. 1).
12. CHITRE, R. G., DESAI, D. B., and RAUT, V. S.—The Nutritive Value of Pure Bred Strains of Cereals and Pulses, Part I. Thiamine, Riboflavin and Nicotinic-acid Contents of 107 Pure Bred Strains of Cereals and Pulses (Vol. 43, No. 4).
13. CHITRE, R. G., DESAI, D. B., and RAUT, V. S.—The Nutritive Value of Pure Bred Strains of Cereals and Pulses. Part II. The Effect of Storage on Thiamine, Riboflavin and Nicotinic Acid in Cereals and Pulses (Vol. 43, No. 4).
14. DAS, N. N., DAS GUPTA, S. R., MUKHERJEE, K. L., and WERNER, G.—Hæmodynamic Effects of *Rauwolscine* (Vol. 43, No. 1).
15. DE, H. N.—Studies on Cholinesterase. Effect of Thiamine on the Serum Cholinesterase Activity of Human Blood (Vol. 43, No. 1).
16. DE, N. K.—A Fluorometric Method for Vitamin A Assay (Vol. 43, No. 3).
17. DE, H. N., DATTA, P., and MANKAD, P. N.—Studies on Riboflavin Metabolism with Typical Indian Dietaries (Vol. 43, No. 3).

18. DIKSHIT, P. K., and RANGANATHAN, S.—Studies of Carotene Metabolism in Mammals. Part I. Experiments with Goats (Vol. 43, No. 3).
19. DIKSHIT, P. K., and RANGANATHAN, S.—Studies in Carotene Metabolism in Mammals. Part II. Experiments with Albino Rats (Vol. 43, No. 3).
20. DIKSHIT, P. K.—Effect of Vitamin D on Parathyroidectomized Rats (Vol. 43, No. 4).
21. D'SILVA, C. B., and MENGI, D. L.—A 'Single Dose' Rabies Vaccine for Dogs (Vol. 43, No. 3).
22. DUDANI, A. T.—Use of Guinea-pig Serum for Identification of Rough Strain of *Vibrio choleræ* (Vol. 43, No. 3).
23. DUDANI, A. T., and AHUJA, M. L.—A Simple Method for the Preparation of Dried Antibiotic Sensitivity Disks (Vol. 43, No. 3).
24. FATTERPAKER, P., MARFATIA, U., and SREENIVASAN, A.—Rôle of Folic Acid and Vitamin B_{12} in Transmethylations. Part I. Formation of Creatine *in vitro* and *in vivo* (Vol. 43, No. 1).
25. FATTERPAKER, P., MARFATTIA, U., and SREENIVASAN, A.—Rôle of Folic Acid and Vitamin B_{12} in Transmethylations. Part II. A Sparing Effect by Formate or Methanol on the Impairment of Creatine Metabolism in Folic Acid Deficiency (Vol. 43, No. 2).
26. FATTERPAKER, P., MARFATIA, U., and SREENIVASAN, A.—Rôle of Folic Acid and Vitamin B_{12} in Transmethylations. Part III. An Influence of Folic Acid on the Methylation of Nicotinamide (Vol. 43, No. 2).
27. FATTERPAKER, P., MARFATIA, U., and SREENIVASAN, A.—Rôle of Folic Acid and Vitamin B_{12} in Transmethylations. Part IV. Non-participation of Folic Acid in the Biosynthesis of Methyl Compounds from Precursors (Vol. 43, No. 2).
28. GADGIL, R. K., and SHAH, S. N.—Human Schistosomiasis in India. Part II. Infection of Snails with *Schistosoma hæmatobium* (Vol. 43, No. 4).
29. GOPALAN, C., SRIKANTIA, S. G., and VENKATACHALAM, P. S.—Electrocardiographic Changes in Severe Malnutrition (Vol. 43, No. 1).
30. GOPALAN, C., and RAMALINGASWAMI, V.—Kwashiorkor in India (Vol. 43, No. 4).
31. LILY, G., MANI, G. S., BALASUBRAMANIAN, S. C., and BASU, K. P.—Growth-promoting Value of Milk and some Indigenous Milk Products (Vol. 43, No. 2).
32. LILY, G., MANI, G. S., BALASUBRAMANIAN, S. C., and BASU, K. P.—Supplementary Value of Milk and some Indigenous Milk Products to a Poor Rice Diet (Vol. 43, No. 2).
33. LIVER DISEASES SUB-COMMITTEE.—A Critical Review of Infantile Cirrhosis of the Liver (Vol. 43, No. 4).
34. LUNDGREN, NILS, P. V., RAMANATHAN, N. L., SEN GUPTA, A., and CHAKRAVARTI, H. S.—Electrical Conductivity and Specific Gravity of Small Volumes of Human Sweat and their Relations to the Salt Concentration (Vol. 43, No. 1).

35. MALAKAR, M. C., BANERJEE, S. N., and GUHA, B. C.—Studies on the Effect of Canning and Storage on the Nutritive Value of some Common Vegetables. Part I. Changes in Ascorbic Acid in Cabbage (Vol. 43, No. 1).

36. MANI, G. S., LILY, G., BALASUBRAMANIAN, S. C., and BASU, K. P.—Composition and Nutritive Value of some Indigenous Milk Products (Vol. 43, No. 2).

37. MAZUMDER, R., and BANERJEE, S.—Studies on the Mechanism of Primary Alloxan Hyperglycemia and Hypoglycemia in Rabbits (Vol. 43, No. 4).

38. PHANSALKAR, S. V., and PATWARDHAN, V. N.—Utilization of Animal and Vegetable Proteins: Urinary Nitrogen Excretion in Absorptive and Early Post-absorptive Phases (Vol. 43, No. 2).

39. RAJ, H., and JOSHI, N. V.—Amino-acid Composition of the Milk of Indian Buffaloes. Part I. Essential Amino-acid Composition of Total Proteins and Protein Fractions (Vol. 43, No. 4).

40. RAJ, H., and JOSHI, N. V.—Essential Amino-acid Composition of the Milk of Indian Buffaloes. Part II. α and β-Casein Components (Vol. 43, No. 4).

41. RAMANATHAN, M. K.—Biochemical Changes in the Serum in Nutritional Œdema Syndrome (Kwashiorkor) (Vol. 43, No. 3).

42. RAMANATHAN, M.K., VENKATACHALAM, P.S., SRIKANTIA, S. G., and GOPALAN, C.—A follow-up Study of Hundred Cases of Nutritional Œdema Syndrome (Kwashiorkor) (Vol. 43, No. 2).

43. RAO, SHANTA, S., and RAO, S. S.—Action of Crystalline Trypsin on Egg Albumin. Part I. Action on Native Heat-denatured and Formalinized Crystalline Egg Albumin (Vol. 43, No. 4).

44. RAO, SHANTA, S., and RAO, S. S.—Action of Crystalline Trypsin on Egg Albumin. Part II. Paper-chromatography Analysis of Amino Acids released during the Action of Trypsin on Heat-denatured Egg Albumin (Vol. 43, No. 4).

45. SACHDEV, J. C.—Vitamin B_{12} and Fatty Livers in Pancreatic Duct Ligated Rats (Vol. 43, No. 1).

46. SETH, G. K., DE, S., and BHASKARAN, T. R.—Treatment and Disposal of Liquid Wastes from the Lac Industry (Vol. 43, No. 3).

47. SHAH, S. N., and GADGIL, R. K.—Human Schistosomiasis in India, Part I. The Study of Snails (Vol. 43, No. 4).

48. SHAH, S. N., and GADGIL, R. K.—Human Schistosomiasis in India. Part III. Note on the Clinical Survey of the Endemic Focus (Vol. 43, No. 4).

49. SHUKLA, R. C., and MUKERJI, B.—Studies on the Chromatophorotropic Hormone of the Pituitary. Part V. The Melanocyte (Dendritic Cell) and its System in Human Skin (Vol. 43, No. 3).

50. SINGH, H. D., and BANERJEE, S.—Studies on the Effect of Germination on the Availability of Iron in some Common Indian Pulses (Vol. 43, No. 3).

51. SOMESWARA RAO, K., RAMANATHAN, M. K., TASKAR, A. D., and PHANSALKAR, S. V.—The Failure of Vitamin B_{12} to promote Growth in Undernourished Indian Children (Vol. 43, No. 2).
52. SRINIVASAN, P. R., and PATWARDHAN, V. N.—The Effect of Protein Deficiency on (*a*) some Liver constituents and (*b*) Enzymes in Liver, Pancreas and Blood Plasma in Albino Rats (Vol. 43, No. 1).
53. TILAK, H. V.—Investigation into the Problem of Late Abortions from Four to Seven Months of Pregnancy (Vol. 43, No. 4).
54. VALANJU, N. N., and SOHONIE, K.—The Studies in the Nutritive Value of Bombay Fish. Part I. Study of Fats of Different Fishes (Vol. 43, No. 2).
54. VARKI, G., VENKATACHALAM, P. S., SRIKANTIA, S. G., and GOPALAN, C.—Study of Birth Weights of Infants in Relation to the Incidence of Nutritional Œdema Syndrome (Kwashiorkor) (Vol. 43, No. 2).
55. VENKATACHALAM, P. S., SRIKANTIA, S. G., GOPALAN, C.—Changes in Body Composition during Nutritional Rehabilitation (Vol. 43, No. 4).
56. VIJAYARAGHAVAN, P. K.—The Hæmopoietic Activity of some Food Proteins (Vol. 43, No. 4).
57. WAHI, P. N.—Pulmonary Calcification and Sensitivity to Tuberculin (Vol. 43, No. 1).
58. WAHI, P. N., and GUPTA, K.—Effect of Radiation on Vaginal Cytology in Cervical Carcinoma (Vol. 43, No. 3).

B. *In the Indian Journal of Malariology.*

1. DAVID, A., and NAIR, C. P. Observations on a Natural (Cryptic) Infection of Trypanosomes in Sparrows (*Passer domesticus* Linnæus) Part I. Susceptibility of Birds and Mammals to the Trypanosomes. (Vol. 9, No. 2).
2. JASWANT SINGH, and MOHAN, B. N.—Susceptibility of *Culex* (Culex) *bitæniorhynchus* Giles, 1901, to *Plasmodium relictum* but not to *Plasmodium gallinaceum* and *Plasmodium falciparum* (Vol. 9, No. 2).
3. MOHAN, B. N.—Comparative Susceptibility of some *Ædes* Mosquitoes to *Plasmodium gallinaceum* (Vol. 9, No. 2).
4. MOHAN, B. N.—Comparative Experimental Infection in *Anopheles fluviatilis* and *Anopheles stephensi* (type) with *Plasmodium falciparum* Welch, 1897 (Vol. 9, No. 2).
5. MOHAN, B. N.—Experimental Studies on Reproductive Capacity of *Anopheles fluviatilis* and *Anopheles stephensi* (type) after Exposure to Sublethal Doses of D.D.T. in Different Stages of Gonotrophic Cycle. (Vol. 9, No. 2).
6. MOHAN, B. N.—Sporogony Cycle of Malaria Parasites in Resistant and non-resistant Strains of Mosquitoes after Exposure to D.D.T. (Vol. 9, No. 4).
7. RAJINDAR PAL, SHARMA, M. I. D., KRISHNAMURTHY, B. S., and GABBA, J. L.—Field Studies on the Comparative Effectiveness of D.D.T., B.H.C. and Dieldrin Residual Sprays against Anopheline Mosquitoes (Vol. 9, No. 1).

IX. LIST OF PAPERS ON WORK DONE UNDER THE AUSPICES OF THE INDIAN COUNCIL OF MEDICAL RESEARCH PUBLISHED DURING 1955.

MALARIA.

BASU, B. C., and GHOSH, S. M.—Studies on the Bionomics of *Phlebotomus argentipes* Ann. and Brun. *Bull. Cal. Sch. Trop. Med.*, **III,** p. 24, 1955.

BASU, B. C.—Some observations on *P. berghei* Infection. *Bull. Cal. Sch. Trop. Med.*, **116**, 1955.

BASU, P. C., NAIR, C. P., and DAVID, A.—A Natural Trypanosome (sp.) Infection in Indian Quail (*Columix communis*). *Bull. Nat. Soc. Ind. Mal. Mos. Dis.*, **3,** No. 6, 1955.

DAS GUPTA, B., RAY, H. N., DUTTA, B. N., and SEN GUPTA, P. C.—Cytochemical Study of *Plasmodium gallinaceum* in *Ædes ægypti.* A Preliminary Note. *Bull. Cal. Sch. Trop. Med.*, **3**(**1**)**,** p. 28, 1955.

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DAS GUPTA, B., and RAY, H. N.—The Changes in the Stomach Epithelium after Blood Feed in *Ædes ægypti.* A Preliminary Note. *Bull. Cal. Sch. Trop. Med.*, **3**(**3**)**,** p. 119, 1955.

DAS GUPTA, B., and RAY, H. N.—Histochemical Observations on the Salivary Glands of *Ædes ægypti. Bull. Cal. Sch. Trop. Med.*, **3**(**4**)**,** p. 169, 1955.

DAS GUPTA, B., and RAY, H. N.—The Effect of Gamma Benzene Hexachloride on the Malpighian Tubules of *Ædes ægypti. Bull. Cal. Sch. Trop. Med.*, **3**(**4**)**,** p. 169, 1955.

DAVID, A.—Microfilaria in Grey Partridge (*Francolinus pondicerianus*). *Bull. Nat. Soc. Ind. Mal. Mos. Dis.*, **3,** No. 2, 1955.

DAVID, A., and NAIR, C. P.—Observations on a Natural (Cryptic) Infection of Trypanosomes in Sparrows (*Passer domesticus* Linnæus). Part I. Susceptibility of Birds and Mammals to the Trypanosomes. *Ind. J. Mal.*, **9,** No. 2, 1955.

JASWANT SINGH, and MOHAN, B. N.—Susceptibility of *Culex* (Culex) *bitæniorhynchus* Giles, 1901, to *Plasmodium relictum* but not to *Plasmodium gallinaceum* and *Plasmodium falciparum. Ind. J. Mal.*, **9,** No. 2, 1955.

KRISHNAN, K. S.—Incrimination of Malaria Vector in Vindhya Pradesh. *Bull. Nat. Soc. Ind. Mal. Mos. Dis.*, **3,** No. 3, 1955.

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